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SOIL SCIENCE

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GEORG WIEGNER

UNIV. OF MICH.

Georg Wiegner
1883-1936

"Young friend," Prof. Georg Wiegner once said to one of his assistants, "when I am dead you will have to write my obituary, and remember, my creed is that of duty, as taught by Kant." With a joke, the young assistant changed the subject but promised to write a paper on "Professor Wiegner" with the reservation that he could write as he pleased. This incident was promptly forgotten until ten years later, when, on April 14, 1936, a cable, "Professor Wiegner died this morning," brutally reminded the present writer of his promise.

CURRICULUM VITAE

Georg Wiegner was born in Leipzig, Germany, on April 20, 1883, the third of twelve children. His youth was clouded with hardship and poverty. He told me once that because he could not afford to buy a Latin dictionary he had to copy one by candle light. With the aid of far-seeing teachers and friends he was permitted to attend the Realgymnasium, which he finished as Primus Omnium in 1902. In the same year he enrolled in the University of Leipzig and graduated in chemistry in 1906. His thesis on "Metastable conditions between solid and gaseous substances" already revealed the keen thinker and successful investigator. From October 1906 to October 1907 he served in the army and then accepted a position as assistant chemist at the Agricultural Institute of the University of Göttingen. His experience at this famous center of learning proved to be a turning point in his life because here he was drawn to the great Zsigmondy, co-inventor of the ultramicroscope, who "captured" Wiegner for colloid chemistry. Soon Wiegner handled the ultramicroscope like no one else. In 1911 he became Privatdozent for Agricultural Chemistry at Göttingen, and in 1913 he married Katharina Claus. In the same year he was offered a professorship in agricultural chemistry at the Federal Institute of Technology in Zürich, Switzerland, which, fortunately for the Swiss, he accepted. Here began his ascending path, which in the course of 23 years led him to international fame. The World War temporarily interrupted Wiegner's climb. He was wounded before the English lines, but in 1916 returned to his friends in Zürich. He remained faithful to his chosen country and declined attractive calls to the Universities of Bonn-Poppelsdorf, Hohenheim, and Munich (as successor to Ramann).

Wiegner became an honorary member of many learned societies. On account of family traits Wiegner never expected to live long. With this in view he carefully trained his body with exercise and sports and appeared a picture of health. Suddenly on April 2 of this year a serious stomach opera-

tion became necessary, which, quite unexpectedly, he did not survive. He was 53 years old. He had no children.

THE LAST UNIVERSAL AGRICULTURAL CHEMIST

Liebig applied chemistry to agriculture, and for him the soil, the plant, and the animal formed a closed and inseparable cycle. Today agricultural chemistry is becoming so highly differentiated into soil phases, plant chemistry, animal nutrition, dairy chemistry, fertilizer production, etc., that it becomes increasingly impossible for a human being to master them all. Wiegner's genius appeared just at the right moment to accomplish this feature for the last time in the history of science. The extent of his vast field of learning is evident from the courses he regularly taught: quantitative agricultural chemistry; soil science; fertilizers, their production and use; animal nutrition, introductory and advanced; chemistry of milk and milk products; chemistry of feeding stuffs; agricultural technology; colloid chemistry. He lectured on soil science every semester, either for agricultural students or for foresters. On certain days he lectured five hours. Every Monday afternoon he took his class on a field trip in coöperation with the Crops Department.

The surprising thing is that Wiegner not only delivered his lectures in admirable form, but that he was an international authority in every field he taught. Soil science, nutrition, dairy science, and colloid chemistry all claim him as a leader in their respective disciplines. He was made an honorary member of the International Society of Soil Science (1935); he was given an honorary degree in veterinary science for his work in nutrition (1933); and the International Colloid Society awarded him the Laura Leonard medal for outstanding research in theoretical and applied colloid chemistry (1934).

His interest oscillated somewhat irregularly among the various branches of agricultural chemistry, as revealed by his publications. He wrote about a dozen papers on pure physical and colloid chemistry, ten papers on colloid chemistry of milk, nearly forty papers on soils, and over thirty papers on animal nutrition. He published well over a hundred articles, not including the dissertations of his students and the papers of his coworkers.

Wiegner started out in physical chemistry. At Göttingen his chief, Professor Fleischmann, aroused his interest in milk chemistry, and Wiegner published a series of papers on milk sugar, milk serum, specific gravity of milk, and degree of dispersion of milk constituents. Professor Baragiola once remarked that with Wiegner a new era had begun in dairy chemistry.

During his assistantship Wiegner took agricultural courses and became fascinated with chemical problems in soils, particularly with base exchange. His splendid contributions in the domain of soils will be dealt with in a separate chapter.

Professor Lehmann introduced Wiegner into the science of nutrition, but the young Privatdozent postponed special investigations until his professorship at Zürich. As a consequence of food scarcity in Switzerland during the War,

Wiegner attacked the problems of digestability of war bread and chemically treated foodstuffs. His equipment was most primitive. He had a few rabbits and one ram. His mode of approach and the results obtained, however, were so impressive that the Swiss farmers gained confidence in Wiegner and in 1925 provided him with a well-equipped institute of animal nutrition. He trained an excellent staff of assistants and studied both practical and theoretical phases of animal nutrition. His contributions to the theory of nutrition, the mathematical formulation of feeding effects, and the energy evaluation of foods on the basis of meat and fat production have attracted world-wide attention. Much emphasis was placed on methods of conservation of silage and on the merits of various forms of hay making. In his pasture studies Wiegner had a fine opportunity to combine his knowledge of soils, of grass quality, and of animal nutrition.

In 1926 Wiegner published a laboratory manual on quantitative agricultural chemistry, dealing with analyses of soils, fertilizers, feeds, milk, and wine (Borntraeger, Berlin, 1926, 348 pages).

If Liebig was the father of agricultural chemistry, Wiegner was the last of the generation as far as supreme command of the entire field is concerned. In looking back upon Wiegner's life and impressive production, many points of contact between Liebig and his admirer, Wiegner, appear. There is great similarity not only in the types of problems studied but also in their viewpoints on the value of science in agriculture. Like Liebig, Wiegner disliked the utilitarian intruders in agricultural science who worshipped the god of production. "Judging a piece of research merely by its immediate practical value may be excused for an experiment station," Wiegner once said, "but at the University the agricultural chemist should assume a broader outlook and strive for fundamental truth."

WIEGNER AS A SOIL SCIENTIST

Wiegner was not a "soils man" as we understand the term in this country. He had no agricultural background and had never plowed a field. His contact with the soil was indirect. He was a chemist, and the soil was an object through which colloid chemistry might be studied and applied. He was not interested in the soil as such. In this respect he was a counterpole to Marbut.

In spite of his handicap—or perhaps because of it—Wiegner has influenced the development of modern soil chemistry more than any other contemporary soil scientist, Gedroiz perhaps excepted. If soil science is to come of age Wiegner will have played no small part in it. For a century soil scientists have unscrupulously borrowed from the pure sciences, as a matter of course, without thinking of repaying anything. Wiegner started to pay back. His sedimentation apparatus was welcomed in many scientific and industrial laboratories. He showed that base exchange is not a mere soils problem; rather it is a general principle in nature. Biochemists and chemists in general, have slowly begun to realize the truth of Wiegner's contention. Wiegner's principles of coagula-

tion, their theory and application, are a permanent ornament to soil science. If we want to have a soil science with emphasis on *science* we must do more than help the farmer: we must contribute to the general store of knowledge, discover laws, invent methods, and produce theories which also fertilize other sciences. Wiegner has blazed the trail in this direction. So has Marbut. Both thought highly of each other.

Wiegner's first contribution to soil science has become a classic. It was published in the *Journal für Landwirtschaft*, volume 60, 1912, under the title "On base exchange in soil." Wiegner gave credit to the work of Thomas Way and showed that Liebig had obscured the issue. According to Wiegner base exchange is a chemical reaction as far as equivalency is concerned, but otherwise has all the characteristics of an adsorption reaction. It follows Freundlich's isotherm.

During the War, in the trenches, Wiegner conceived his well-known sedimentation apparatus. For decades soil scientists had been content with the isolation of certain soil fractions, such as sand, silt, and clay, but Wiegner, as a colloid chemist, wanted to know the entire distribution curve. Like the Sven Oden balance, the Wiegner tube gives a continuous distribution diagram and for certain purposes is superior to other methods. The apparatus has been modified by several investigators.

In 1916 Wiegner gave a lecture on soils before the Society of Natural Sciences in Zürich, as a result of which he published a little book entitled "Soil and soil formation from a colloid chemical standpoint" (Steinkopf, Dresden, sixth unrevised edition, 1931). This brief but excellent exposition at once made Wiegner popular and internationally known. Here was something new. The common idea of the soil's containing mysterious colloids was changed to the concept of the soil as a disperse system. Clay formation was explained as a mutual flocculation of negative, colloidal silicic acid and positive, colloidal aluminum and iron hydroxides.

From 1922 to 1927 Wiegner was particularly active in soil research. In a paper "Dispersity and base exchange" (Zsigmondy volume of the *Kolloid Zeitschrift*, 1925) he laid the cornerstone of modern research on soil colloids and on certain phases of pure colloid chemistry as well. In the introduction he wrote:

> In my opinion base exchange is a problem which embraces more than the special field of soil science. It is a part of general dispersoid chemistry. Base exchange is of fundamental importance not only for the cation content of clay, permutite, and soil—in which soil scientists are particularly interested—but is also determinant for the qualitative and quantitative properties such as stability, swelling, etc., and for the variations of properties (peptization and flocculation) of most colloidal systems (colloidal sulfur, AgCl, As_2S_3 dye stuffs, proteins, etc.).

In the same paper he introduced into soil chemistry the concept of hydration of ions in order to explain the lyotropic series. Subsequent detailed experiments on base exchange with permutite demonstrated the fruitfulness of this

approach. Coagulation studies, viscosity measurements, heat of wetting, and and swelling data, all proved the significance of the nature of the adsorbed ion.

In connection with the sedimentation apparatus Wiegner started to re-investigate the mechanism of coagulation. He distinguished between orthokinetic and perikinetic flocculation and, with the aid of the mathematical physicist H. Müller, put the Smoluchowski equations on a broader foundation. Smoluchowski's formulas apply only to monodisperse systems (e.g., colloidal gold, or Sven Oden sulfur), whereas the new equations comprise polydisperse systems such as soil.

About 1930 Wiegner published the suspension effect which was to change radically our notions of soil acidity and alkalinity. He showed that the adsorbed H ions affect the electrode, an observation previously made and explained by Bradfield. Wiegner extended the same concept to adsorbed OH ions. An excellent summary of his viewpoints has been published in English in the *Journal of the Society of Chemical Industry*, volume 50, pp. 55–62, 65–71, 103–112, 1931, under the titles "Coagulation" and "Some physico-chemical properties of clays."

Wiegner took an active though indirect part in the soil survey of Switzerland and directed extensive studies on the destruction of concrete pipes in soils.

Professor Wiegner once remarked that in science every man eventually returns to his first love. He entered soil chemistry by the way of base exchange and left it by the same avenue. His last publication was on "Ionic exchange and structure," which appeared in the *Transactions of the Third International Congress of Soil Science*, volume III, p. 5–28, 1936. A comparison between Wiegner's first and last papers affords a good illustration of the impressive development of a research problem in the hands of a great sculptor in science.

WHAT MADE WIEGNER A GREAT SCIENTIST?

Three important features have contributed to Wiegner's success: he possessed a wealth of original ideas; he was thoroughly trained and was *gründlich* in his work; he knew how to present his results.

The first attribute cannot be acquired. Wiegner was given a full share of it. He had new ideas about everything. No matter what was discussed, be it science, philosophy, politics, America, dictators, or what not, he surprised his listeners with new angles, original viewpoints, and unsuspected cause and effect relationships. He was particularly prolific in the production of scientific ideas and he saw problems everywhere. At one time he had nearly twenty associates and kept every one of them at a high pitch with daily discussions and stimulating interpretations. He was a born general in the field of research.

Wiegner went through the old German educational mill—hard work in school and more work at home; memorizing and analyzing. It cleared the head and gave a solid foundation. Wiegner left the Realgymnasium as the highest ranking student and he forgot nothing of what he had learned. As the "Old Professor," he still beat everyone in multiplication and division (no machines

needed); complicated mathematical formulas seemed ever ready up his sleeve. He remembered the classics better than did his students who came directly from the school bench. At the university he had grand teachers, the greatest in German science, Nobel prize winners left and right. He saw into their workshops and learned his lesson. "Good training in the past is not sufficient," Wiegner asserted, and he kept up with modern developments. At the height of his fame, he attended Debye's courses on electrolytes and took courses in advanced mathematics. He used to say that a chemist has to relearn chemistry about every ten years. He did it—at night. Like Willstätter, he could have said: "For thirty years I have heard the midnight bells at my desk."

The Germans are thorough and systematic, and Wiegner was a typical example. He checked and rechecked his measurements, he calculated backwards and forwards. I remember the time when I collaborated with him in preparing a textbook on agricultural chemistry. We wished to discuss pH measurements. What is the proper soil-water ratio? He made me go through all the German, French, English, Dutch, and Scandinavian literature and tabulate what others suggested. Then I had to test the formulas in the laboratory. Naturally, it took many days to write a page, but then the material stood the test.

Wiegner was an artist also. True, he cared little for music, painting, or sculpture, yet he was a born poet and at times liked to compose. He could not help but reveal this trait in his scientific papers. He never published a mere progress report, a little contribution, or a note to the editor. He presented a finished whole, a dramatic account of the problem, thesis and antithesis, attractive descriptions. His scientific papers read like novels and his publications always made a hit. No one will forget his speeches. When Wiegner rose, the atmosphere became electrified, people listened most intently, and a roaring applause followed his finale. On such occasions we assistants were particularly proud of our master and admired him accordingly.

Wiegner has trained a formidable array of research workers. Not counting the pure colloid chemists and nutrition men, such soil scientists have been associated with Wiegner, for longer or shorter periods, Albareda, R. V. Allison, Barnette, Beutelsbacher, Blom, Bradfield, Cernescu, Gallay, Georgescu, Gessner, Jenny, Kawashima, Marshall, A. Meyer, K. W. Müller, Oedelien, Pallmann, Renold, E. W. Russell, Salminen, Scherf, Steward, Souviron, Szigeti, Tendeloo, Tokuoka, Tuorila, Weisz. The list is by no means complete.

WIEGNER AS A TEACHER AND FRIEND

Certain people are of the opinion that a good research man necessarily is a poor teacher, or, *vice versa*, that a good teacher makes a poor scientist. Anyone acquainted with Wiegner will gladly testify to the contrary. Wiegner was a great scientist as well as a gifted teacher. He never had a course in education, yet his lectures were a thrill to his students. They did not miss a single lecture, and this is the crucial test in an educational system in which attendance is not enforced.

Whoever entered Wiegner's laboratory as a co-worker found the door open to his private life. He gave himself unmasked and freely expressed his opinions about the outer world. By this he gained the confidence of his associates, and we told him our personal troubles and asked for advice, which he freely gave. Looking back to the twenties, I am now ashamed to recall how much of his precious time we consumed with our own petty affairs, even going so far as to read to him passages from Schopenhauer, who at that time had our attention. Wiegner did not stop us or complain; in fact, he lived with us because he had remained young. Like all great men and really busy people he seemed to have time for everything and everybody.

Wiegner's body is gone. His discoveries will become commonplace, his theories revised, and his methods changed beyond recognition; but his spirit is preserved in the hearts of his students and will be passed on from generation to generation.

HANS JENNY.

PLANTS IN THE SUB-FAMILY CAESALPINIOIDEAE OBSERVED TO BE LACKING NODULES[1]

O. N. ALLEN AND ETHEL K. ALLEN

University of Hawaii

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The symbiotic relationship between leguminous plants and the root nodule bacteria is commonly recognized by the formation of nodules on the root systems. The majority of plants attributed this characteristic to date are members of the sub-family Papilionoideae, although nodule formation is not uncommon in the Mimosoideae. Many exceptions are known to occur in the Caesalpinioideae.

Cassia chaemacrista L., added to the cowpea group by Burrill and Hansen (7) in 1917, is the only species of the sub-family Caesalpinioideae contained in the cross-inoculation groups compiled by Walker (23) and by Fred, Baldwin, and McCoy (11). Recently additional species, *Cassia mimosoides* L., *Cassia pilifera* Vog., and *Hymenaea courbaril* L., have been added to the same group (2). Rhizobia cultures isolated from nodules of each of these species inoculated cowpea plants. It was possible, however, to make the reciprocal cross with only two of the species, *Cassia mimosoides* L. and *Cassia pilifera* Vog., with cowpea bacteria, since seeds of *Hymenaea courbaril* L. could not be obtained.

Various observations on the lack of nodule formation on species of the Caesalpinioideae have been reported. A list of such species appears in table 1.

During the past 5 years a rather extensive survey of nodule formation has been conducted on tropical leguminous plants growing in the Territory of Hawaii. Special attention was directed to the species of the sub-family Caesalpinioideae in an attempt to understand better the extent of nodulation. Table 2 contains the data obtained from the survey.

All of the plants examined were growing under natural conditions in forest or field soil. Seeds of practically all of the species were imported sometime in the distant past from other tropical areas. The plants have become well established, many now existing as large trees. Examination consisted of two procedures: (*a*) the removal of the soil from most of the root mass and (*b*), in many instances, the planting of seeds of the species in several lots of island soil known to contain Rhizobia representative of the cross-inoculation groups. The latter is true with all species listed in table 2 of which more than four plants were examined. It is questionable that data obtained from such a

[1] Published with the approval of the director as Technical Paper No. 91 of the Experiment Station of the Pineapple Producers' Cooperative Association, University of Hawaii.

TABLE 1

Members of the sub-family Caesalpinioideae reported to be lacking nodule formation

SCIENTIFIC NAME (COMMON NAME)	AUTHORITY
Cercis canadensis L. (Redbud or Judas tree)	Lachmann (16), 1858; Buckout (5), 1889; Harrison and Barlow (13), 1906; Leonard (17), 1925
Cercis siliquastrum L.	Clos (9), 1893
Gymnocladus dioica (L.) Koch (Kentucky coffee tree)	Buckout (6), 1890; Leonard (17), 1925; Harrison and Barlow (13), 1906
Cassia barclayana Sw.	Clos (9), 1893
Cassia marylandica L. (Wild or American senna)	Leonard (17), 1925
Cassia medsgeri Shafer (Medsger's wild senna)	Leonard (17), 1925
Cassia tora L. (Low senna, coffee-weed)	Leonard (17), 1925
Cassia occidentalis L. (Coffee senna, styptic-weed)	Leonard (17), 1925
Cassia corymbosa Lam.	Leonard (17), 1925
Cassia bicapsularis L. (Stiber-bush)	Leonard (17), 1925
Cassia emarginata L.	Leonard (17), 1925
Cassia laevigata Willd.	Leonard (17), 1925
Cassia tomentosa Wall.	Leonard (17), 1925
Cassia artemisoides Gaudlich	Leonard (17), 1925
Gleditsia triacanthos L. (Honey locust)	Nobbe, Schmid, Hiltner and Hotter (21), 1891; Harrison and Barlow (13), 1906; Leonard (17), 1925
Caesalpinia brevifolia Baill.	Naudin (20), 1894
Caesalpinia exostemma Moss. & Sesse	Leonard (17), 1925
Caesalpinia melanocarpa Griseb.	Leonard (17), 1925
Caesalpinia pulcherrima (L.) Sw. (Barbados pride, dwarf poinciana)	Leonard (17), 1925
Ceratonia siliqua L.	Leonard (17), 1925

TABLE 2

*Tropical species of the sub-family Caesalpinioideae observed to be lacking nodules**

SCIENTIFIC NAME (COMMON NAME)	HABITAT	KIND OF PLANT	NUMBER EXAMINED	AGE OF PLANTS EXAMINED
Saraca declinata Miq.	Sumatra	Tree	3	About 3 years
Saraca thaipingensis Cantley	China and Malay	Tree	3	About 3 years
Saraca indica L. (Asoka, sorrowless tree)	East Indies and Malay	Tree	3	About 3 years
Schotia brachypetala Sond.	South Africa	Shrub	15	1–5 months
Schotia latifolia Jacq. (Elephant hedge or bean tree)	South Africa	Tree	3	About 3 years

* The authors are deeply indebted to Mr. E. L. Caum, assistant botanist of the Experiment Station of the Hawaiian Sugar Planters' Association, for his making available the majority of the species listed in this table and for his generous aid in their classification.

TABLE 2—*Concluded*

SCIENTIFIC NAME (COMMON NAME)	HABITAT	KIND OF PLANT	NUMBER EXAMINED	AGE OF PLANTS EXAMINED
Tamarindus indica L. (Tamarind)	Tropical Africa	Tree	16	12 seedlings 1–4 months, 4 trees about 7 years
Bauhinia binata Blanco	India	Small tree	20	3 months to 1 year
Bauhinia monandra Kurz.	Burma	Small tree	15	3 months to 1 year
Bauhinia variegata Linn. (Napoleon's hat, mountain ebony)	East Indies and Burma	Tree	38	3 to 5 months
Cassia fistula Linn (Indian laburnam, golden shower)	Tropical Asia	Tree	12	2 months to 2 years
Cassia grandis L. f. (Horse cassia, liquorice tree, pink shower)	South America	Tree	22	1 month to 2 years
Cassia laevigata Willd. (Smooth senna)	Tropical America	Shrub	40	1 month to 2 years
Cassia glauca Lam.	India	Small tree	50	1 month to 5 years
Cassia nodosa Burch.-Ham. (Pink and white shower)	Tropical Asia	Tree	18	2 months to 2 years
Cassia occidentalis Linn. (Negro coffee, stinking weed, common cassia)	Tropical America	Shrub or herb	Several hundred	1 month to 2 years
Cassia moschata H. B. & K.	South America	Tree	4	About 4 years
Cassia alata L. (Candle-bush, ringworm bush, golden candle stick)	Tropical America	Shrub	25	1 to 8 months
Cassia siamea Lam. (Iron-wood of East Indies, Kassod tree, Siamese senna)	East Indies	Tree	16	1 to 5 months
Caesalpinia pulcherrima (L.) Sw. (Barbados pride, Barbados flower fence)	Pantropic	Shrub	14	3 months
Caesalpinia sappan L. (Sappan wood, sapang)	Tropical Asia	Tree	4	1 to 4 months
Caesalpinia crista L. (Brazilletto, redwood, nickernut)	West Indies	Shrub	8	1 to 3 months
Caesalpinia sepiaria Roxb. (Wait-a-bit, Mysore thorn)	Tropical Asia	Shrub	10	1 to 6 months
Pellophorum inerme (Roxb.) Naves (Yellow poinciana)	Malasia	Tree	16	1 to 6 months
Mezoneurum kauaiense (Mann) Hillebr. (Uhiuhi)	Hawaiian Islands	Tree	8	1 to 12 months
Delonix regia (Boj.) Raf. (Flamboyant, flame tree, royal poinciana)	Madagascar	Tree	23	1 to 12 months

survey warrant the assertion that these plants listed do not form nodules, but the facts do signify, on the other hand, that under these conditions of growth and habitat and by the methods of examination employed, nodules were not present.

DISCUSSION

Why certain leguminous plants bear nodules and others do not has provoked many attempts at explanation. Nobbe, et al. (21) were among the first to suggest that the physical character of the root hairs of several species in the sub-family Caesalpinioideae inhibited nodule formation. McDougall (19) expressed the same opinion as a result of his study on the thick-walled root hairs of species in the genus Gleditsia and related genera. Joshi (15) proposed the idea that root nodule bacteria might exist in the root tissues of certain leguminous plants, yet without root nodules being formed. A lower virulence of the specific bacteria or a slower reaction of the plant to the stimulus given by the bacteria was offered as an explanation for the lack of typical nodule formation. In 1926 Friesner (12) and Feher and Bokor (10) reported on the roots of *Gleditsia triacanthos* L. cylindrical swellings which contained bacteria and functioned as root nodules. Questionable evidence (15) has been offered showing that Rhizobia may exist in parts of the leguminous plant other than in nodules upon the roots, although a positive case was not found in *Cassia tora* L., when various histological procedures, involving combination fixing and staining methods were employed (1).

Whether the specific microörganism is present or not will continue to constitute the requisite proof with the majority of species to which lack of nodule formation is ascribed. As noted in table 1, *Cercis canadensis* L. was reported by Buckout (5), Harrison and Barlow (13), and others to be lacking nodules. In 1909 Buchanan (4) reported physiological studies on *Cercis* bacteria from nodules. Later reports tend to support the absence of nodules on this species. In 1889 Buckout (5) considered *Mimosa pudica* L. a non-nodulating plant. In 1932 Fred, Baldwin, and McCoy (11) reported that nodules sometimes occurred on roots of *Mimosa pudica* L. growing under greenhouse conditions. Later, in 1934, Leonard (18) reported the isolation of bacteria from the nodules of *Mimosa pudica* L. under conditions different from those commonly employed for other root nodule bacteria, and proposed the placing of this species in a separate cross-inoculation group. Recently Carroll (8) showed that nodule bacteria from a related species, *Mimosa strigillosa*, inoculated *Vigna sinensis* L. A somewhat similar case has been noted in the literature with respect to *Trifolium alexandrianum* L. In 1933 Sarkaria (22) reported this species as a non-nodulating plant under conditions of culture in India, although the species was placed in the clover group by Burrill and Hansen as early as 1917. Later the failure of the species to form nodules was explained by the fact that the root nodule bacteria were absent from the soil.

The outstanding fact remains, however, that some factor induces the penetration of the root nodule bacteria into leguminous root systems, and nodule formation results. Leonard (17) has epitomized the situation in the following manner: "As a matter of fact, the problem extends beyond the Leguminosae, for it has not been determined why, with few exceptions, bacteria root nodules

do not occur on non-leguminous plants, or, stated in another way, why they are peculiar to leguminous plants only."

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PHOSPHORUS DEFICIENCY IN CITRUS

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In order to add a so-called complete fertilizer, phosphorus is frequently added to soils in which citrus trees are grown. It is usually considered that, although not subject to appreciable loss from leaching, phosphorus is largely fixed in relatively unavailable forms and that the principal opportunity of the roots to absorb phosphorus occurs during a rather brief period subsequent to the time of application. Little importance appears to be attached to the continuous daily absorption from low concentrations of phosphorus obtained from so-called insoluble soil compounds or to the solubility and other physical or chemical soil effects that result from the use of organic manures and cover crops. Although these organic materials are rich in soluble phosphorus, only the nitrogen and organic matter ordinarily are given consideration, because they determine the cost. The relatively high phosphate concentration found in most citrus soils would appear to necessitate correspondingly large applications of nitrogenous fertilizers and may be involved in the relation of certain of the minor elements to healthy growth in citrus.

Because of the relatively low phosphorus requirement of citrus (the leaves and fruit contain considerably less phosphorus than either potassium or nitrogen) there is the greater uncertainty regarding the need of applying phosphorus to the soil. Indicator plants may be of value in detecting excesses or deficiencies of an element for trees, but the use of such plants is most limited, the best indicator plant being either the trees themselves or replants close by.

No case of proved phosphorus deficiency with citrus in the field has as yet been clearly demonstrated; in fact, the symptoms of phosphorus deficiency have not as yet been described. Interest rather has long been focused on the relation of excessive phosphorus absorption to the phenomenon of mottle-leaf.

This paper deals with the deficiency phase of phosphorus nutrition in citrus. It is shown that citrus trees can withstand a deficiency of phosphorus for a considerable time, apparently without injurious effects, and can absorb in a few days when phosphorus is relatively soluble, a supply that suffices for long periods.

METHODS

Plants in soil and sand cultures

Citrus plants were grown in Sierra loam soil outdoors in 12-gallon earthenware jars without drainage. Quartz sand (obtained from the Philadelphia Sand

Company) was used in cultures likewise conducted outdoors in 12-gallon glazed earthenware jars and also in galvanized iron cans 20 inches in diameter and 24 inches deep. Ottawa silica sand that was acid-treated and washed with distilled water was used in experiments in the glasshouse in 2-gallon glazed earthenware jars with drainage and in 6-liter capacity Swedish white enameled pans without drainage.

The following salts were used in preparing the culture solution: potassium nitrate, magnesium sulfate, sodium chloride, calcium nitrate, potassium dihydrogen phosphate, iron tartrate, boric acid, and manganese sulfate. The culture solution had the following composition (expressed as parts per million):

Na	K	Ca	Mg	NO_3	Cl	SO_4	PO_4	Fe	Mn	B	Total
7	185	159	54	718	10	216	105	1	0.1	0.1	1,455.2

Solution cultures

Solution culture experiments were conducted in Swedish white enameled pans of capacities varying from 4 to 18 liters and in pails of similar material but of 9-liter capacity. The culture solution was the same as that employed for the sand cultures and, unless otherwise stated, was not aerated nor was the pH controlled.

The citrus plants used in the soil, sand, or solution cultures consisted of budded trees or of rooted leafy-twig cuttings including various combinations of such cuttings as regards scion and stock.

Preparation and analysis of plant material

At the conclusion of an experiment the plants were photographed and then subdivided into the various portions, such as leaves, twigs, and bark. The samples were rapidly washed with distilled water, wiped clean, and dried in a large well-ventilated oven at 65°C. The materials were later ground either by hand in a tinned mill or by means of a motor-driven Wiley mill, after which the samples were redried.

Chemical analysis

The methods employed for the determination of the various constituents were those used by Haas and Bliss (8). Phosphorus in the dry matter was determined by the ashing method employed by Howk and DeTurk (11) followed by the molybdenum blue colorimetric method essentially as used by Truog and Meyer (15). The various phosphorus fractions in the dry matter were determined by the method of DeTurk, Holbert, and Howk (2).

EXPERIMENTAL

The chief objective in the culture studies was to grow citrus in sand and solution cultures in which phosphate was omitted and to note the effect, not only on the appearance of the trees, but also upon their chemical composition.

Haas (6) has reported the effect of various concentrations of phosphate on the growth and composition of lemon cuttings produced in culture solutions.

The solution cultures on phosphorus deficiency, to be reported later in this paper, were a valuable supplement to the sand cultures because of the more rigid control possible in the glasshouse. Although the sand cultures grown outdoors were well covered, they were nevertheless the recipients of phosphorus-containing dusts, which, if they do not reach the sand immediately, eventually are washed there by the rains.

TABLE 1

Phosphorus content of various portions of citrus trees

TREES	PHOSPHORUS, AS PER CENT OF DRY MATTER, IN				
	Leaves	Shoots	Bark of trunk	Bark of roots	Young roots
Lisbon lemon cuttings grown 2 years in phosphate-deficient sand cultures	0.0688	0.0300	0.0388	0.0538	0.0694
Lisbon lemon tree on sweet-orange stock; grown 2 years in soil in nursery	0.4125	0.3063	0.0575	0.0850	0.1450
Valencia orange trees on sweet-orange stock; grown 2 years in phosphate-deficient sand cultures	0.0550	0.0425	0.0400	0.0463	0.0625
Valencia orange tree on sweet-orange stock; grown 2 years in soil in nursery	0.6500	0.2750	0.0875	0.0825	0.2000

	Bark of 1928 shoots	Bark of 1927 shoots	Bark of 1928 trunk elongation	Remainder of trunk		Bark of main tap roots	Bark of side roots
				Bark of upper half	Bark of lower half		
Rough lemon seedlings 3 years old from seed; grown in soil in nursery until October, 1928	0.0825	0.0800	0.1200	0.1100	0.0925	0.1175	0.1063

Trees grown in phosphate-deficient sand cultures and in soil

Three Lisbon lemon cuttings and three Valencia orange trees budded on sweet orange as rootstocks were transplanted bare root (washed with tap and then with distilled water) from soil in the nursery to sand cultures in galvanized iron cans. The trees were grown in these sand cultures for approximately 2 years, during which time the cultures received a complete nutrient solution so modified as to substitute nitrate for phosphate. At the conclusion of the experiment the trees had a basal trunk caliper of approximately 1 to 1½ inches, and showed symptoms typical of phosphate deficiency.

The percentages of phosphorus in the dry matter of the various portions of these young trees are given in table 1. The leaves of the trees in the phosphate-deficient sand cultures contain approximately 0.05 to 0.07 per cent of phosphorus in the dry matter; the leaves of trees in soil contained many times

this amount. The soil was markedly deficient in nitrogen, and, as Haas (7) has shown, the leaves of trees in such soils contain higher percentages of phosphorus. The shoots and the trunk and root bark of the phosphate-deficient cultures contained less phosphorus than did the leaves or young roots.

One of the lemon trees in the phosphate-deficient cultures produced several lemons. These fruits contained 0.06 per cent of phosphorus in the dry matter, approximately that found in the leaves and roots.

A group of Rough lemon seedling trees 3 years of age from seed were obtained in the nursery and were used for the determination of the percentage of phosphorus in the bark of young trees. The bark of the shoots (table 1) contained smaller percentages of phosphorus than that of the trunk. A gradient in the

TABLE 2

Phosphorus fractions of Valencia orange and Lisbon lemon trees grown in phosphorus-deficient sand cultures and in soil

VARIETY	PORTION USED	PHOSPHORUS AS PER CENT OF DRY MATTER					
		Inorganic		Organic		Phospholipid	
		Phosphorus-deficient sand cultures	Soil	Phosphorus-deficient sand cultures	Soil	Phosphorus-deficient sand cultures	Soil
Valencia orange	Mature leaves	0.0210	0.4625	0.0195	0.2900	0.0065	0.0188
	Mature shoots		0.1550		0.0825		0.0067
	Bark of trunk	0.0120	0.0600	Trace	0.0330	0.0034	0.0052
	Bark of main root	0.0138	0.0725	Trace	0.0183	0.0053	0.0060
	Young rootlets	0.0188	0.1358	0.0145	0.0359	0.0036	0.0088
Lisbon lemon	Mature leaves	0.0300	0.3750	0.0112	0.1600	0.0053	0.0215
	Mature shoots	0.0133	0.1900	Trace	0.0750	0.0022	0.0101
	Bark of trunk	0.0100	0.0575	Trace	0.0250	0.0047	0.0057
	Bark of main root	0.0133		Trace		0.0042	
	Young rootlets	0.0200	0.0950	0.0110	0.0370	0.0034	0.0074

phosphorus content occurred in the trunk bark, with the lowest percentage near the base of the trunk.

The shoots and bark of citrus trees grown in phosphorus-deficient sand cultures are the first portions in which the percentages of organic phosphorus are reduced to a trace. The data in table 2 suggest that organic phosphorus within the tissues may be used by citrus when conditions for obtaining inorganic phosphorus are desperate.

The percentages of inorganic and organic phosphorus shown in table 2 decrease from the leaves to the shoots and trunk and increase from the main root to the young rootlets. The percentages of the various phosphorus fractions are generally highest in the leaves, even after a prolonged period of phosphorus starvation.

In table 3 are presented the percentages of inorganic, organic, and phospholipid phosphorus in leaves from trees grown in artificial solution cultures. When phosphate was present for only a few days at intervals of several weeks, the percentage of inorganic phosphorus in the leaves of Valencia orange leafy-twig cuttings was 0.1293, with no accompanying symptoms of phosphorus deficiency. In the absence of phosphorus in the culture solution, symptoms were developed to a marked degree, and the percentage of inorganic phosphorus in the dry matter of Valencia orange leaves was 0.0175 and in Eureka lemon leaves 0.0388. Drastic reductions in the percentages of organic and phospholipid phosphorus resulted when phosphorus was omitted from the culture solution.

TABLE 3

Phosphorus fractions in citrus leaves from trees grown with deficient phosphate in artificial cultures

CULTURES	VARIETY	PHOSPHATE CONCENTRATION IN THE CULTURE MEDIUM	PHOSPHORUS AS PER CENT OF DRY MATTER		
			Inorganic	Organic	Phospholipid
Valencia orange cuttings as scions on Rough lemon cuttings as stocks; grown from March 2, 1933, to June 18, 1934	Valencia orange	Solution cultures (control); 105 p.p.m. PO_4 for a few days at intervals of several weeks	0.1293	0.0550	0.0084
Valencia orange cuttings as scions on Eureka lemon cuttings as stocks; grown from March 2, 1933, to June 18, 1934	Valencia orange	Solution cultures; no PO_4	0.0175	0.0088	0.0028
Eureka lemon cuttings; grown from April 11 to December 26, 1933; cultures maintained at pH 4.5 with HNO_3	Eureka lemon	Solution cultures; no PO_4	0.0388	0.0078	0.0038

The Eureka lemon roots of the grafted cuttings in which Valencia orange leafy-twig cuttings were used as the scions (table 1) contained 0.0100, 0.0108, and 0.0035 per cent, respectively, of inorganic, organic, and phospholipid phosphorus in the dry matter. These results approximate those found for the Valencia orange leaves of the same trees (table 3) and point to an equilibrium in the phosphorus distribution of such phosphate-starved plants. This same condition is also shown in the percentages of organic phosphorus in the leaves and roots of phosphorus-deficient sand cultures with lemon trees (table 2).

In view of the results of Heck and Whiting (10) and others, who found that organic phosphorus was utilized by certain plants, and of the results of Pierre and Parker (14), who found that organic phosphorus was not available for plant growth, it was considered desirable to ascertain whether or not lemon cuttings could utilize organic phosphorus.

Many experiments were carried on with lemon cuttings grown in nonaerated culture solutions containing no phosphate, but to which phytin was added. The cuttings in most cases showed a growth response. Agitation of the solution at intervals, by means of a stirring rod, to insure contact of the roots with the phosphorus, greatly improved the growth.

In experiments with aerated cultures, lemon cuttings were grown without phosphate until every mature leaf had fallen and only a single long shoot with phosphorus-deficient leaves at the tip remained. Phytin was then added to the solution of such cultures in 6-liter pans. Within a few days, many of the lateral buds and many of the roots began to grow. Phytin appears to be of value as a source of phosphorus for citrus when the phosphorus supply is deficient.

Symptoms of phosphorus deficiency in leaves

One phase of the symptoms of phosphorus deficiency in Valencia orange leaves is shown in plate 1, figure 1, in which the chlororphyll is seen to fade in certain areas. Another phase is indicated in plate 1, figure 2, by the burning of the blade at the tip, which without careful diagnosis may be confused with injury caused by high chlorine; by the burning of the blade in various locations other than at the tip, as shown in the leaf on the right in the upper row; by the reduction in leaf size; and by the dull brownish green color and lack of luster.

In plate 1, figure 3, are seen some of the symptoms typical of phosphorus deficiency in lemon leaves taken from cuttings grown in solution cultures in the glasshouse. There is no uniformity in the location of the leaf-burn, and abscission soon follows the burning. The subsequent new leaves, when mature, were dwarfed, brittle, and burned, and possessed a characteristic dull brownish green color. A deficiency as indicated by such symptoms was evident in the leaves of all Lisbon lemon cuttings grown in culture solutions containing up to and including 1 p.p.m. of phosphate. At this highest concentration the symptoms were evident in a minor way only in a few leaves. The roots of phosphate-deficient cuttings also were affected and will be described later.

With the phosphate-deficient Valencia orange and Lisbon lemon sand cultures grown outdoors were similar cultures in which walnut trees (*Juglans regia*) were grown. Likewise, in aerated solution cultures in the glasshouse, walnut seedlings were grown without phosphate. No symptoms of any deficiences produced under controlled conditions have as yet been described for walnut trees. Plate 1, figure 4, shows the burning or death of tissue as a result of a phosphate deficiency in sand cultures. In the solution cultures the burning frequently was very severe. Walnut trees utilize large amounts of phosphorus, and these preliminary results suggest the similarity of the effects of a phosphate deficiency on leaves of citrus and walnut trees.

In the glasshouse at first, Swedish enamel ware pans were used for sand cultures without drainage. The Ottawa silica sand was acid-treated and

washed with distilled water prior to being used. Solutions were renewed by periodically flooding the sand with either distilled water or culture solution or with both, followed by decantation of the excess solution.

The growth of Lisbon lemon cuttings in the three cultures lacking phosphate far exceeded that in the other cultures, as is shown in plate 1, figure 5. This experiment proved to be a starting point in an investigation regarding the concentration of phosphorus in relation to growth.

In a second experiment with Lisbon lemon and Valencia orange cuttings, the (Philadelphia) silica sand was acid-treated and washed with distilled water. Some cultures received a complete solution containing 105 p.p.m. of phosphate, while others received a solution lacking phosphate. The omission of phosphorus was accompanied by an increased growth of the tops, but after a time the leaves of the cultures receiving no phosphorus showed burning typical of phosphate deficiency.

The leaves of the control cultures had to be wiped free of spiders every little while, although the spiders failed to become established on the leaves of adjacent phosphate-deficient cultures. Phosphate-deficient leaves are more brittle, and perhaps the leaf surfaces are more difficult to penetrate. No trouble at any time from scale insects was encountered in these cultures lacking phosphate. Haas (5) has shown that citrus scale insects on fruits contain relatively large amounts of phosphorus.

In plate 2 are shown transverse sections of Lisbon lemon leaves obtained from cuttings grown in a complete culture solution and in such a solution lacking phosphate. The cells in the palisade tissue are narrow and elongated (pl. 2, fig. 1) when phosphorus is abundant and are nearly square (pl. 2, fig. 2) when phosphate is deficient in the culture solution. The cells of the phosphorus-deficient section reveal very few contents as compared with those of the control section. This condition, together with changes in cell structure and toughness or brittleness of the leaves, may be a factor inhibiting the action of the mite commonly called "red spider." A decreased rate of cell division, an increased cell wall thickening, and the occurrence of cortical cells similar in shape to these palisade cells have been described as occurring in phosphate-deficient tomato plants by Kraybill (12) and by Eckerson (3).

The early symptoms of phosphorus deficiency in lemon cuttings are seen in plate 3, figure 1. There is an increased shoot growth, which is one of terminal elongation accompanied by an almost total absence of lateral shoots. The leaves absciss from the lowermost portions of the new shoots produced by the original cuttings. The leaves of the original cuttings remain attached long after the symptoms of deficiency appear in the young leaves. The younger leaves usually are smaller than the older ones, although there is no gradation of leaf size from the base of a shoot to the tip. Frequently a series of small leaves may be followed in the next growth cycle by fewer but considerably larger leaves. Leaves at the stage shown in plate 3, figure 1 *B*, begin to show burned areas as previously described, and although the leaves feel brittle and

stiff, many of them droop at the petiole in a direction parallel to the shoot axis.

The symptoms of phosphate deficiency of lemon cuttings grown in non-aerated cultures and the recovery therefrom will next be considered in connection with advanced stages of phosphorus deficiency. Plate 3, figure 2 *A* shows an extreme case of such deficiency in lemon cuttings grown in a culture solution lacking phosphate and aluminum. The lower or mature leaves have fallen, the roots have become dark in color and have ceased growing, and the lateral bud growth has remained suppressed.

It is seen in plate 3, figure 2 *B*, that the lateral buds are alive and able to grow once phosphorus becomes available. The cuttings in *B* were grown without phosphorus or aluminum in the culture solution until the growth resembled that shown in *A*. Then 105 p.p.m. of phosphate but no aluminum was used in the culture solution for a few days at intervals of several weeks. Note the recovery in *B* that resulted from the use of phosphorus after a long period of phosphorus starvation. The pH of the solution was not controlled and at times ranged between 7 and 8.

The first effects of the addition of phosphate were seen in the tops. Within a week or so after phosphate was used in the culture solution, the new terminal growth ceased to consist of small, dull, brownish green leaves. The new leaves were large and healthy, and abscission ceased for the time being. The old roots were short, brown, and stubby, but otherwise in good condition, and ultimately began to grow. Very few, if any, of the lateral buds started growth until sometime later.

The retained leaves apparently first manufactured food to supply their own needs and then secondarily those of the roots and lateral buds. The new roots did not appear until months after the tops had shown the recovery evident in plate 3, figure 2 *B*. The delayed root recovery suggests that, in the absence of aluminum to precipitate or retain phosphorus in or on the root tissue during phosphate starvation and early recovery, a marked depletion of phosphorus and carbohydrate reserves possibly may take place in the roots. Haas (6) has shown the relation of the sugar content of lemon leaves to the phosphate concentration in the culture solution.

When phosphate-deficiency symptoms occur in lemon cuttings grown without phosphate in the culture solution, the phosphorus contents of the dry matter of the leaves fall in the range from 0.0363 to 0.0736 per cent. The varied locations of burn in phosphate-deficient leaves are indicative of considerable variation in the phosphorus distribution in leaves or of the degree of sensitivity of different tissues to phosphate deficiency.

Symptoms of deficiency were overcome in part only by spraying phosphorus-deficient Valencia orange trees grown in sand cultures and lemon cuttings grown in solution cultures, with a mixture containing phosphate. For this purpose a solution was used consisting of 10 gm. of $CaH_4(PO_4)_2$, 5 gm. of $Ca(OH)_2$, and 100 cc. of water containing blood albumin spreader. No benefit resulted when the $Ca(OH)_2$ was omitted.

Heretofore, the discussion has been confined largely to the symptoms of phosphorus deficiency in lemon trees. Attention is now directed toward such a deficiency or an excess in relation to the growth of Valencia orange and pomelo cuttings. The absence of data regarding the phosphate nutrition of orange and grapefruit cuttings has greatly retarded the successful growing of such trees in culture solutions. With 105 p.p.m. of phosphate in the non-aerated or aerated culture solution (pl. 4, fig. 1) the cuttings start growth, but after the first new cycle the original leaves gradually fall off, and the growth of the roots comes to a standstill.

Many orange and grapefruit cuttings were discarded because of their failure to grow. By experimentation it was found that the use of 105 p.p.m. of phosphate for a few days just prior to the renewal of the culture solution at intervals of several weeks and, as shown elsewhere, the use of 5 to 10 p.p.m. of aluminum as citrate when phosphate was absent permitted healthy, vigorous growth.

In plate 4, figure 2, are seen Valencia orange cuttings as scions on Rough lemon cuttings as stocks after being grown in a phosphate-free culture solution containing no aluminum. The growth of the roots came to a standstill while terminal growth was still being made by the shoots. The leaves were of a dull brownish green color and many of the lowermost ones were falling.

The cuttings illustrated in plate 4, figure 3, show a striking contrast in growth when phosphate was deficient. The continued use of 105 p.p.m. of phosphate produced a healthy top but a most abbreviated root system (pl. 4, fig. 3 *A*). When, however, phosphate was omitted from the culture solution (pl. 4, fig. 3 *B*) the growth of the roots showed improvement for a time, but gradually the oldest leaves were lost until the tops of the cuttings presented a thin appearance.

The growth of pomelo leafy-twig cuttings was improved (compare with plate 4, figure 1 *B*) by using 105 p.p.m. of phosphate in the culture solution for only a few days at intervals of several weeks, as shown in plate 5, figure 1. Note the coarse, heavy type of root characteristic of pomelo cuttings. Additional improvement in growth was later brought about by the use of 5 to 10 p.p.m. of aluminum in the culture solution during the interval of several weeks when phosphate was omitted.

The effect of advanced stages of phosphate deficiency in pomelo cuttings will next be considered. In plate 5, figure 2, are seen pomelo cuttings as scions on Eureka lemon cuttings as stocks. In *A*, the original leaves still remain attached, while most of the oldest new leaves have fallen and there is a suppression of growth of the lateral buds. This inhibition of the growth of lateral buds suggests a possible relation of phosphorus to the delayed foliation of deciduous trees. In *B*, all of the leaves have fallen except the very youngest. The roots are coarse and, although their growth is at a standstill, in neither case have they broken down.

The leaves are a dull brownish green color. The roots (in plate 5, figure 2 *A*, and in many other cuttings among these cultures) consisted of thick, coarse

laterals with few fine roots. Many of these lemon cuttings used as stocks have produced roots of the coarse type usually found on rooted pomelo cuttings. This may be due in part to the active vegetative growth of the pomelo variety and the availability of large supplies of organic materials because of the extensive leafy growth. This effect of scion on stock also has been referred to by Halma (9), who found that the Eureka lemon scion, when grafted to twigs of sour orange, effected a change in the inherent form of the root system of the latter.

TABLE 4

Phosphorus content of various portions of young Valencia orange and pomelo trees grown in solution cultures deficient in phosphorus

VARIETY	DURATION OF THE EXPERIMENT	CULTURE SOLUTION	PHOSPHORUS, AS PER CENT OF DRY MATTER, IN		
			Leaves	Shoots	Roots
Valencia orange cuttings as scions on Eureka lemon cuttings as stocks	March 2, 1933, to June 18, 1934		0.0419	0.0306	0.0463
Valencia orange cuttings as scions on Lisbon lemon cuttings as stocks	June 7, 1933, to June 18, 1934	PO_4 absent	0.0563	0.0313	0.0500
	June 7, 1933, to June 20, 1934		0.0425*	0.0256	0.0525
Valencia orange cuttings as scions on Rough lemon cuttings as stocks	June 7, 1933, to May 25, 1934		0.0556	0.0325	0.0438
	June 7, 1933, to June 20, 1934	105 p.p.m. PO_4 present	0.1500	0.2150	.....
Pomelo cuttings on Eureka lemon cuttings as stocks	March 13, 1933, to June 18, 1934	PO_4 absent	0.0469	0.0288	0.0500
	August 29, 1931, to June 19, 1934		0.0425	0.0338	0.0475

* Phosphorus deficiency very advanced; considerable leaf abscission.

Table 4 presents data in regard to the percentage of phosphorus in the various portions of Valencia orange and pomelo cuttings grown in phosphate-deficient culture solutions. When the phosphate deficiency symptoms were obvious, the percentages of phosphorus for the leaves and roots were approximately equal and those for the shoots were intermediate. This phosphorus equilibrium in leaves and roots may indicate a standstill condition of growth. When 105 p.p.m. of phosphate was used in the culture solution the percentage of phosphorus in the shoots exceeded considerably that in the leaves.

Lemon cuttings grown in a nonaerated culture solution containing 105 p.p.m.

of phosphate made fair growth for a time, after which the growth of the root system came to a standstill, as shown in plate 6, figure 1 *A*. The leaves frequently were chlorotic, notwithstanding the liberal use of ferric tartrate in the culture solution. When this decline was evident, traces of many elements not ordinarily used in culture solutions (4) were added but without benefit. This suggested that either the phosphate was preventing the traces of elements from functioning or that the phosphate itself was excessive and a factor in bringing about injury to the roots of nonaerated cultures.

The culture solution used for the growth of the cuttings (pl. 6, fig. 1 *A*) was subsequently modified by omitting the phosphate and adding the minor elements. The removal of phosphate was accompanied by a marked improvement in the growth of the roots and tops, as shown in *B*.

It is still possible that the actual decrease in the phosphate concentration in the culture solution was not solely responsible for the improvement in the growth. The removal of phosphate permits the traces of elements to remain better in solution, and these may then have brought about the improvement. Results thus far obtained have shown a beneficial effect of a temporary depletion of the phosphate supply and the further benefit of aluminum during such phosphate depletion in unaerated culture solutions. Haas (6) has described the excellent growth obtainable when 105 p.p.m. of phosphate is used in a thoroughly aerated culture solution. The results indicate a possible relation of aeration with phosphate absorption and health in roots. Aeration apparently becomes of greater importance as the concentration of phosphate in the culture solution increases.

Phosphorus deficiency in lemon cuttings in relation to silica

Many investigators (1, 13, and others) have shown the interrelation between silica and phosphorus in plants and are in agreement that silica may partially replace phosphorus. This relation of silica to phosphorus was tested in preliminary experiments by growing lemon cuttings in a culture solution lacking phosphorus and then adding silicic acid to the solutions in which a number of the cuttings were grown.

Plate 6, figure 2, shows the differences in growth produced by the addition of silicic acid to culture solutions lacking phosphorus. Other Lisbon lemon cultures, grown in a similar manner but for a short period (February 18 to March 22, 1935), showed the better growth in the presence of silicic acid when phosphate was absent. This growth response was independent of the changes in pH brought about by the addition of the silicic acid to the culture solution because the pH of all cultures in these experiments was maintained at approximately the same favorable values, 6 to 6.5.

SUMMARY

A study was made of the deficiency phase of phosphorus nutrition in citrus by the use of soil, sand, and solution cultures. The effect of such a deficiency

was noted on the appearance of the trees and also on their chemical composition.

The leaves of trees in phosphate-deficient sand cultures contained approximately 0.05 to 0.07 per cent of phosphorus in the dry matter, whereas the leaves of trees in soil (low in nitrate nitrogen) contained several times these percentages. The shoots and the trunk and root bark of the phosphate-deficient cultures contained smaller percentages of phosphorus than the leaves or young roots. Lemon fruits of phosphate-deficient cultures contained 0.06 per cent of phosphorus in the dry matter, a value approximating that found in the leaves and roots.

The shoots and the bark are the first portions to have the percentages of their organic phosphorus fraction reduced to a trace when phosphorus is omitted from the culture solution. Organic phosphorus within the tissues or in the culture solution may be used by citrus when conditions for obtaining inorganic phosphorus become desperate. The percentages of phospholipid phosphorus are highest in the leaves even after a prolonged period of phosphorus starvation.

Symptoms of phosphorus deficiency in citrus leaves are variable and may consist of a fading of the chlorophyll, a burning of the blade in various locations, a reduction in leaf size, or a change to a dull brownish green color without luster.

A phosphorus deficiency brings about changes in the shape and contents of the cells of lemon leaves. There is an increased shoot growth, but it is merely one of terminal elongation accompanied by an almost total absence of lateral shoots. In severe cases the leaves not only burn but feel brittle; they droop at the petiole in a direction parallel to the shoot axis and later absciss. Inhibition of the growth of lateral buds can be overcome by the addition of phosphate to the culture solution.

The growth of Valencia orange and pomelo leafy-twig cuttings was improved by using 105 p.p.m. of phosphate in the unaerated culture solution for only a few days at intervals of several weeks. This method of supplying phosphorus was superior to the continuous presence of lower but still somewhat high concentrations of this element.

In the absence of phosphorus in the culture solution, silica appeared to be of benefit.

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PLATE 1

FIG. 1. Valencia orange leaves from trees grown in sand cultures outdoors in large galvanized iron containers 20 inches in diameter and 24 inches deep. Phosphate was omitted in the culture solution and resulted in the loss of chlorophyll.

FIG. 2. Valencia orange leaves from trees in sand cultures that received a culture solution lacking phosphate. The leaves in the upper row show the reduction in leaf size and the tip and side burn found on leaves of trees grown in galvanized iron containers 20 inches in diameter and 24 inches deep. The lower row of leaves was obtained from trees in sand cultures in 12-gallon earthenware pots. The two leaves on the left in the lower row show gum formation on the under side. The leaves in both rows have a dull brownish green color and a lack of luster.

FIG. 3. Leaves of lemon cuttings as affected by phosphorus in solution cultures: Lower left, healthy dark green leaf from a cutting grown in a solution containing 105 p.p.m. PO_4; remaining leaves from cuttings grown in solutions containing no phosphate. Note the nonuniformity of the location of the leaf burn.

FIG. 4. Dead areas in leaves of walnut trees (*Juglans regia*) grown in sand cultures that received a culture solution lacking phosphate. This condition was frequently found along the veins as shown in the two leaves on the left, although scattered dead areas also were found, as shown in the two leaves on the right. There is no uniformity in the location of the burned areas. In severe cases the burn may cover the entire leaf.

FIG. 5. Lisbon lemon leafy-twig cuttings in preliminary sand cultures. Left to right: complete culture solution (control); sodium substituted for potassium; magnesium for potassium; calcium for potassium; phosphate omitted, and 10 p.p.m. aluminum added as citrate; phosphate omitted, and 20 p.p.m. aluminum added as citrate; and phosphate omitted. The increased growth of the three phosphate-free cultures over that of the control is clearly indicated.

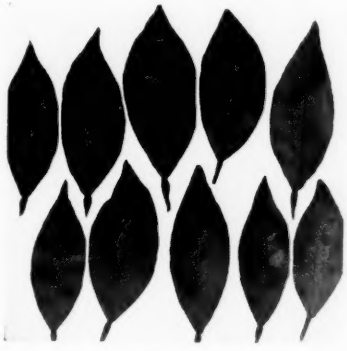

FIG. 1

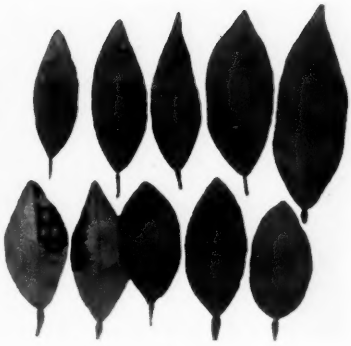

FIG. 2

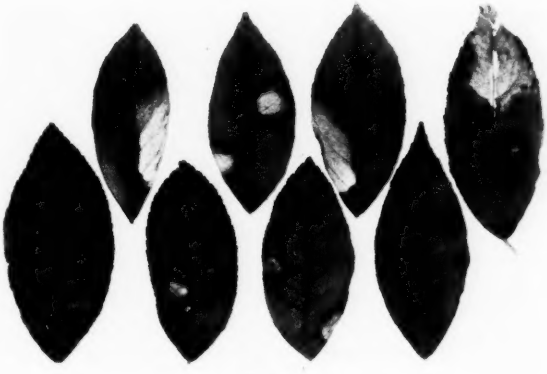

FIG. 3

FIG. 4

FIG. 5

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PLATE 2

TRANSVERSE SECTIONS OF LISBON LEMON LEAVES FROM LEAFY-TWIG CUTTINGS GROWN IN THE GLASSHOUSE

FIG. 1. Leaves from cuttings grown in a complete culture solution.

FIG. 2. Leaves from cuttings grown in a complete culture solution except that phosphate is omitted.

FIG. 1

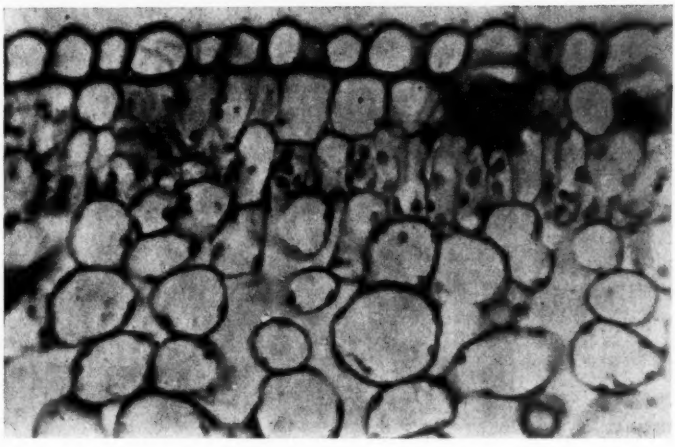

FIG. 2

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PLATE 3

FIG. 1. Early symptoms of phosphate-deficiency in lemon cuttings. *A*, cuttings grown in a culture solution containing phosphate. The internodes are short and the leaves of good size. *B*, cuttings grown in a culture solution without phosphate. The shoots generally remain unbranched and have, therefore, only a terminal growth; the youngest leaves are of reduced size; and the oldest leaves produced while in culture solution absciss after drooping at the petiole for some time in a direction parallel to the shoot axis.

FIG. 2. Recovery of Lisbon lemon leafy-twig cuttings from phosphate deficiency. *A*, effect of growing cuttings from July 14, 1932, to November 9, 1933, in a culture solution lacking phosphate and in which the pH is not controlled. *B*, the state of recovery on November 9, 1933, of cuttings grown from February 19 to June 7, 1933, in a culture solution containing no phosphate or aluminum and grown from June 7 to November 9, 1933, in culture solution in which phosphate (105 p.p.m.) was present only for a few days at intervals of several weeks.

FIG. 1

FIG. 2

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PLATE 4

FIG. 1. *A*, Valencia orange leafy-twig cuttings; *B*, pomelo leafy-twig cuttings. These were grown in a culture solution containing 105 p.p.m. PO_4 beginning on January 8, 1933 (photo May 11, 1934). Most, if not all, of the original leaves on the cuttings have fallen, and growth has come to a standstill. This condition was corrected by the judicious use of phosphate and aluminum.

FIG. 2. Valencia orange leafy-twig cuttings as scions on Rough lemon leafy-twig cuttings as stocks. The cuttings were grown from June 7, 1933, to May 25, 1934, in a culture solution in which phosphate was omitted. Note the loss of the oldest leaves and the exudation of gum from leaf scars. Roots were healthy, although under the conditions they were unable to continue the growth of young rootlets.

FIG. 3. Valencia orange leafy-twig cutting as the scion on Rough lemon leafy-twig cutting as the stock. *A*, grown in nonaerated culture solution (containing 105 p.p.m. PO_4 and five times the normal concentration of calcium nitrate) beginning on June 7, 1933 (photo September 13, 1933). Note the retention of leaves but the dwarfed root system. *B*, grown in nonaerated culture solution (containing no phosphate but containing five times the normal concentration of calcium nitrate) beginning June 7, 1933 (photo September 13, 1933). Note the more sparsely foliated tops and more elongated root system when phosphate was deficient (*B*).

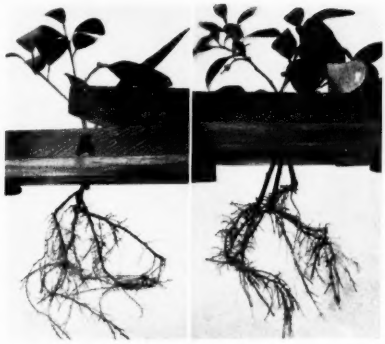

FIG. 1

FIG. 2

FIG. 3

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PLATE 5

FIG. 1. Pomelo leafy-twig cuttings grown in a culture solution containing 105 p.p.m. PO_4. The cuttings grew poorly until the phosphate was allowed to be present for only a few days at intervals of several weeks.

FIG. 2. Advanced stages of phosphorus-deficiency in pomelo leafy-twig cuttings as scions on Eureka lemon leafy-twig cuttings as stocks. The cuttings were grown in a culture solution in which phosphate was omitted. *A*, cuttings grown from August 29, 1931, to December 26, 1933, show the loss of the oldest leaves on the new shoots while the original pomelo leaves still remain attached; *B*, cuttings grown from January 8, 1931, to May 11, 1934, show the loss of all except the youngest new leaves. Root growth is at a standstill. Note the absence of any growth of buds in the axils of leaves that have fallen.

FIG. 1

FIG. 2

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PLATE 6

FIG. 1. Effect of phosphate on the root growth of lemon leafy-twig cuttings grown from July 16, 1932, until September 13, 1933, in nonaerated culture solution containing 105 p.p.m. phosphate. *A*, ineffectiveness of adding to the culture solution 0.1 p.p.m. of the minor elements (4) including zinc and copper; *B*, response following the omission of the phosphate and the addition of 0.1 p.p.m. of the minor elements, as in *A*. The improvement in nonaerated cultures following the omission of phosphate without the addition of the minor elements other than iron, boron, and manganese has been repeatedly shown in other experiments.

FIG. 2. Effect of silica in the absence of phosphate on the growth of Lisbon lemon leafy-twig cuttings. The cuttings were grown in culture solutions from January 7 to March 22, 1935. Left, two cultures from solutions containing 0.1 gm. silicic acid (H_2SiO_3) in 6 liters; right, two cultures from solutions containing no added silica (control).

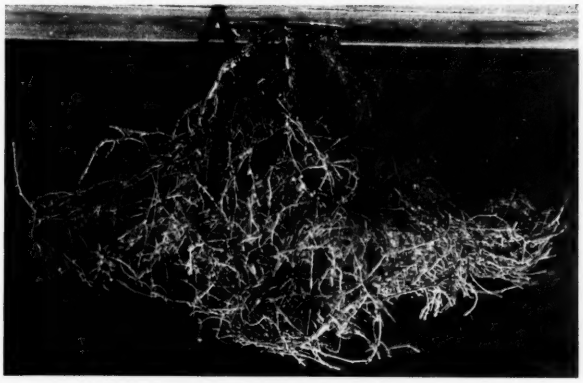

FIG. 1

FIG. 2

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DECOMPOSITION OF LIGNIN BY MICROÖRGANISMS[1]

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The problem of lignin transformation in nature by chemical and biological agencies and the rôle of this process in the formation of humus in soils and in composts, as well as of peat and of coal, have attracted, during the last two decades, considerable attention from chemists, biologists, and agriculturists. The results obtained so far, however, are hardly sufficient to justify a clear conception of the mechanism of lignin decomposition and of the agents concerned in this process.

The accumulated evidence concerning the action of microörganisms upon lignin, in the process of decomposition of plant and animal residues in nature, can be summarized as follows: 1. Lignin is, among chemical complexes, most resistant to attack by fungi, bacteria, and invertebrate animals living in soils, peat bogs, and composts (6, 12, 15, 18). 2. Lignin does not accumulate quantitatively in the same state in which it exists in plant materials but undergoes a slow process of transformation (1, 2, 3, 14). 3. Certain specific groups of microörganisms, found largely among the higher fungi, are capable of bringing about active destruction of lignin, frequently even to a greater extent than that of cellulose and hemicelluloses (4, 5, 21). 4. Lignin is subject to decomposition by these organisms as long as it is present in the fresh or in the partly decomposed plant tissues; once it is prepared in a purified state, it becomes completely resistant to attack even by these organisms. It still remains to be determined whether this is due to the physical or the chemical modification of the lignin complex in the process of its preparation. Lignin produced biologically, as in the decomposition of wood by cellulose-destroying fungi, whereby it is left in a free state, is also resistant to the action of lignin-decomposing microörganisms (3, 11, 17). 5. The effect of lignin upon microbial activities, such as cellulose decomposition, glucose fermentation, and nitrate formation, is not injurious and may even be favorable (19). 6. The chemical nature of lignin in a growing plant varies with the age of the plant,

[1] Journal Series Paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] The authors are indebted to Prof. W. Fuchs, for suggesting the methods of lignin preparation and determination and for collaborating in the chemical part of the investigations; also to Mr. T. C. Cordon, for assisting in the preparation of the alkali lignin and in various chemical analyses.

as shown by an increase in the methoxyl content of the lignin with an increase in the maturity of the plant. This seems to be correlated with the increase in the resistance of the lignin to decomposition, as shown by the fact that the younger the plant the more readily the different plant constituents, including the lignin, undergo decomposition (20). Whether a similar correlation exists between the chemical nature of the lignins in different plants and the rapidity of their decomposition by microörganisms still remains to be determined. 7. Because of its greater resistance to decomposition than is the case of the other plant constituents, the concentration of lignin in the residual material increases with the advance of decomposition of plant residues in soils, composts, and peat bogs (8, 12). Since the decomposed material is usually designated as "humus," the formation and accumulation of humus may be recognized as depending upon lignin accumulation, although lignin may not be the only organic complex contributing to humus formation. 8. The gradual transformation of the lignin noted above consists in (*a*) a loss of methoxyl groups, (*b*) darkening in color, accompanied by absorption of oxygen, (*c*) combination with proteins, (*d*) increase in alkali solubility. As a result of these changes, the alkali-soluble fractions of the humus, commonly referred to as "humic acids," partake of the properties of lignin as modified above, these modifications being characterized largely by an increase in nitrogen content, a lower methoxyl content, and a darker color (7, 13, 14).

In order to study the modifications and changes produced in the lignin molecule by microörganisms capable either of destroying it completely or of modifying only certain parts of it, one must be able to grow these organisms upon isolated lignin. The fact that all previous attempts to grow fungi, which have been found capable of decomposing lignin in natural plant materials or in composted plant residues, upon isolated lignin failed, is believed to be a result of the changes produced in the lignin molecule in the process of its isolation by drastic treatments. Usually either acid lignin or alkali lignin has been employed. When lignin is spread upon a silica gel plate and inoculated with a soil suspension, an abundant population of bacteria, fungi, actinomyces, and invertebrate animals is usually found to develop. However, when these organisms are isolated in pure culture and tested upon the same lignin in agar or liquid media, either they fail completely to attack the lignin or their action is so slow and so limited as to make accurate determinations valueless. An attempt has, therefore, been made to utilize another form of lignin, namely, phenol-lignin (9), as a substrate for the growth of lignin-decomposing organisms. Before the results of these experiments are reported, the destruction of native lignin in a single type of plant, harvested at different stages of growth, is illustrated from a typical experiment.

DECOMPOSITION OF LIGNIN IN OAT PLANTS OF DIFFERENT AGES

Oat plants were harvested from a uniform plot of ground, at three different stages of their development; namely, 59 days old, 86 days old, and 112 days

old or fully mature. At the last stage, only the straw and chaff were used. The results of the proximate chemical analysis of the plants at these three different stages are given in table 1. With an increase in age of plant, there was an increase in hemicellulose, cellulose, and especially lignin content, and a decrease in proteins, mineral substances, and ether-soluble and water-soluble materials.

The three oat plant preparations were air dried and cut into small fractions. Definite amounts, namely, 50-gm. portions of air-dry material containing 6 per cent moisture, were placed in 1-liter Erlenmeyer flasks. The young plant material, containing a higher nitrogen concentration than that required for its normal decomposition (20), received no additional nitrogen. The more mature plant preparations (86 and 112 days old) were treated with 2-gm.

TABLE 1

Chemical composition of oat plants harvested at different stages of growth

Per cent of dry material

	AGE OF PLANT		
	59 days	86 days	112 days
Total nitrogen	2.32	0.96	0.42
Ether-soluble fraction	4.42	2.98	2.26
Cold-water-soluble organic matter	14.85	7.88	3.45
Hot-water-soluble organic matter	4.84	3.23	2.48
Alcohol-soluble fraction	3.07	2.17	1.70
Hemicelluloses	15.25	17.35	19.27
Cellulose	24.55	34.50	39.10
Lignin*	6.70	11.70	15.70
Protein, water insoluble	7.55	2.34	1.70
Ash	9.62	9.05	4.20
Total accounted for	90.85	91.20	89.86

* Acid lignin, ash- and nitrogen-free.

portions of $(NH_4)_2HPO_4$ or with 3-gm. portions of casein; some were left as controls, without nitrogen. All flasks received 2-gm. portions of $CaCO_3$ and 1-gm. portions of K_2HPO_4. The moisture content of the young plant material (59 days) was adjusted to 60 per cent, and that of the older material, to 75 per cent. The flasks were incubated at 28°C. for 47 and 125 days and analyzed (table 2). The results calculated on the basis of 100 gm. of the original dry material are presented in table 3.

The young oat plants decomposed so rapidly that after 47 days half of the total dry material has disappeared; when calculated on an ash-free basis, the percentage loss was even greater. The older the plant material, the slower was the rate of its decomposition. In the same period of time, in the absence of added nitrogen, only about a third of the 86-day-old plants was decomposed and less than a fifth of the mature plant material. The decomposition of the

TABLE 2

Decomposition of oat plants, harvested at different stages of growth

Per cent of residual dry material

Age of Plant ..... days	59		86						112					
Treatment of Material	No nitrogen		No nitrogen		$(NH_4)_2HPO_4$		Casein		No nitrogen		$(NH_4)_2HPO_4$		Casein	
Period of Decomposition ..... days	47	125	47	125	47	125	47	125	47	125	47	125	47	125
Total nitrogen	3.29	3.76	1.60	1.82	3.24	3.92	2.80	2.98	0.70	0.86	2.10	3.63	2.09	3.65
Ether-soluble fraction	3.69	3.30	1.82	1.08	2.12	1.59	2.20	0.55	2.03	1.18	1.77	0.87	1.57	0.53
Cold-water-soluble fraction	15.29	21.92	6.98	8.33	10.31	12.73	9.40	11.34	4.67	5.28	6.92	7.63	6.51	8.97
Hot-water-soluble fraction	5.44	6.80	4.27	6.07	6.80	8.90	6.77	9.66	2.61	4.53	3.80	9.11	4.82	12.86
Hemicelluloses	5.01	1.95	10.70	7.55	7.84	3.66	8.25	4.70	14.03	12.19	9.09	5.18	6.66	5.53
Cellulose	18.87	8.74	21.25	16.23	14.75	7.14	16.54	10.02	29.76	26.09	15.11	7.86	20.08	6.88
Lignin	9.47	7.09	13.35	13.21	14.47	14.89	12.45	12.63	15.91	16.56	20.89	21.08	20.10	16.86
Protein	11.87	15.00	7.02	8.50	13.43	17.88	12.96	13.62	3.23	4.24	8.67	18.76	9.38	18.76
Ash	26.17	33.80	22.15	26.90	27.25	32.50	26.70	31.80	12.30	14.10	21.95	27.30	18.50	24.45
Total accounted for	95.81	98.60	87.54	87.87	96.97	99.29	95.27	94.32	85.54	84.17	88.20	97.79	87.62	94.84

older plants was hastened considerably by the addition of available nitrogen; after 125 days the fully mature oat straw had decomposed to about the same extent as the very young plants when calculated on an ash-free basis. The favorable effect of available nitrogen upon the decomposition of the plant material is largely due to hastening the rapid destruction of the carbohydrate constituents, namely, the cellulose and hemicelluloses. In the case of the lignin, the addition of nitrogen did not have so controlling an influence upon its decomposition during the early stages. After 4 months, however, the amount of lignin decomposed was greatly influenced by the presence of available nitrogen. It is doubtful whether this was due to the direct influence of the nitrogen, since after 47 days very little of the added nitrogen was left in an inorganic form. Possibly, it may have been a result of a secondary influence of the synthesized nitrogenous compounds, which favored the development of lignin-decomposing organisms; it will be shown later that lignin-decomposing microörganisms prefer protein to inorganic salts as sources of nitrogen.

TABLE 3

Total amount of decomposition of oat plants, harvested at different stages of growth

On the basis of 100 gm. of original dry material

AGE OF PLANT	PERIOD OF DECOMPOSITION	SOURCE OF NITROGEN	TOTAL MATERIAL	TOTAL NITROGEN*	HEMICELLULOSES	CELLULOSE	LIGNIN
days	*days*		*gm.*	*mgm.*	*gm.*	*gm.*	*gm.*
59	47	None	48.3	−619	12.66	14.80	1.80
59	125	None	56.3	−692	14.41	20.77	3.63
86	47	None	36.7	+53	10.58	21.05	3.25
86	47	$(NH_4)_2HPO_4$	39.3	+206	12.59	25.56	2.92
86	47	Casein	40.0	−120	12.40	24.58	4.23
86	125	None	37.4	+179	12.62	24.34	3.43
86	125	$(NH_4)_2HPO_4$	62.8	−302	15.99	31.84	6.16
86	125	Casein	52.4	−381	15.11	29.73	5.69
112	47	None	18.5	+151	7.84	14.85	2.73
112	47	$(NH_4)_2HPO_4$	39.7	+46	13.79	29.99	3.10
112	47	Casein	37.6	+44	15.11	26.57	3.16
112	125	None	27.1	+207	10.38	20.08	3.53
112	125	$(NH_4)_2HPO_4$	60.2	+225	17.31	35.97	7.31
112	125	Casein	62.1	+123	17.17	36.49	9.31

* + = gain, − = loss.

The comparatively rapid decomposition of the lignin in this experiment is due to the aerobic nature of the compost. Further, the lignin in oat straw seems to be readily subject to decomposition, since a much smaller amount of humus was left, especially in the presence of available nitrogen, as compared with composts of wheat and rye straw or of deciduous leaves (20).

PREPARATION OF PHENOL-LIGNIN

Phenol-lignin was prepared from the three samples of oat straw by treating the material with 3 parts of phenol containing 0.1 per cent of HCl. The lignin was dissolved in ethyl alcohol and precipitated by diluting and washing with water. The elementary chemical composition of the three samples of phenol-lignin from oat plants, as well as those from lowmoor and highmoor peat is reported in table 4. The carbon content varied within narrow limits, with an average, for the five preparations, of 69.47 per cent, very similar to that of phenol-lignin from spruce wood, which was 69.7 per cent. The nitrogen in the lignin preparations decreased with age of plant. The methoxyl content, however, increased with age of plant; this was true both of the plant itself and of the lignin prepared from it; in the case of the lignin, there was a slight decrease in methoxyl in the fully mature plant. The younger plant did not give so good a recovery of the methoxyl, pointing to a higher concentration of loosely bound methoxyl groups.

TABLE 4

Chemical composition of phenol-lignin from different plant materials and from peats

Per cent of dry material

NATURE OF MATERIAL	YIELD OF LIGNIN	CARBON CONTENT	NITROGEN	METHOXYL CONTENT	
				Original material	Lignin preparation
Oat plant, 59 days	11.6	70.75	0.82	1.71	4.71
Oat plant, 86 days	14.4	68.55	0.38	2.85	9.96
Oat plant, 112 days	27.3	69.69	0.21	3.41	7.95
Lowmoor peat	7.8	68.91	0.73	2.09	5.86
Highmoor peat	18.9	69.47	0.26	0.96	1.96
Oat plant, 112 days*	11.3	64.00	0.63	3.41	13.80

* Alkali lignin.

For the sake of comparison, the analysis of alkali-lignin prepared from the mature oat plant is also reported. Although the yield of this form of lignin is less than that of phenol-lignin, its methoxyl content is considerably higher.

DECOMPOSITION OF PHENOL-LIGNIN BY MICROÖRGANISMS

For the isolation of microörganisms capable of decomposing lignin, silica gel plates were prepared; casein was used as a source of nitrogen, and a dilute solution of certain minerals (K_2HPO_4, $MgSO_4$, $FeSO_4$), as well as $CaCO_3$, was also added. Phenol-lignin was dissolved in a small amount of alcohol, and the solution was added directly to the plates. The excess of alcohol was removed by drying the plates at 60°C. These plates were inoculated with soil and with enrichment liquid cultures containing phenol-lignin. Active development of a number of different fungi and bacteria took place on the plates.

The microörganisms were at first isolated in crude culture and were later reisolated on agar media containing phenol-lignin. They were tested for their capacity to decompose lignin, and only the active forms were utilized.

In the first experiment, phenol-lignin from spruce wood was used. Two-gram portions of the air-dry preparation were dissolved in 50 cc. ethyl alcohol, and the solution was added to 950 cc. water, with constant stirring. One gram of casein, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4 \cdot 7H_2O$, and 0.02 gm. $FeCl_3$ were also added. The medium was distributed into flasks, which were plugged with cotton and sterilized in flowing steam for 1 hour on two consecutive days.

TABLE 5

Decomposition of phenol-lignin by various fungi and bacteria in presence of alcohol

ORGANISM	27 DAYS OF INCUBATION		38 DAYS OF INCUBATION	
	Lignin left	Lignin decomposed	Lignin left	Lignin decomposed
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	153	...	148	...
Bacterium No. 5	20	133	26	127
Bacterium No. 8	15	138	30	118
Fungus No. 1	...	...	118	30
Fungus No. 2	12	141	10	138
Fungus No. 4	...	...	30	118
Fungus No. 7	25	130	12	136
Fungus No. 8	108	45	131	17

TABLE 6

Decomposition of phenol-lignin in alcohol-free medium

ORGANISM	CO_2-LIBERATED	NH_3-LIBERATED	LIGNIN LEFT	LIGNIN DECOMPOSED
	mgm. C	*mgm. N*	*mgm.*	*mgm.*
Control	16.7	...	178	..
Crude bacterium No. 5	42.9	4.7	122	56
Fungus No. 2	33.4	2.6	140	38
Fungus No. 7	41.9	3.8	111	67

Some of the flasks were left as controls, and others were inoculated with several of the cultures found capable of decomposing the lignin. The flasks were incubated at 28°C. for 27 and 38 days and were then analyzed for residual lignin. The method of determining the lignin consisted in extracting the culture with ethyl acetate and removing the extract through a separatory funnel; the extraction was repeated several times, until all the lignin was removed; the extracts were combined, filtered, placed in small weighed bottles, evaporated on a water bath, and dried to constant weight.

The results obtained (table 5) show that all the organisms tested were cap-

able of decomposing the phenol-lignin actively but to a varying quantitative extent. The three most active forms were found to be fungi Nos. 2 and 7 and bacteria Nos. 5 and 8. Different cultures of the same organisms behaved similarly.

A study was now made of the decomposition of phenol-lignin in the absence of alcohol. The alcoholic solution was prepared as before, and the alcohol was removed by evaporation under reduced pressure. The nutrient substances were added, and the solutions were made up to volume and distributed into flasks. These were sterilized, inoculated, and incubated for 46 days. The CO_2 liberated and the ammonia produced (from the casein) were also determined (table 6). The three different organisms tested were found to decompose phenol-lignin also in the absence of the solvent; however, the amounts decomposed were considerably less.

In order to test the effect of another solvent, 5 gm. of lignin was dissolved in 50 cc. acetone, and the solution was suspended in water; the acetone was removed by evaporation, at reduced pressure, and the solution was made

TABLE 7

Decomposition of phenol-lignin in acetone-free medium

ORGANISM	LIGNIN LEFT	LIGNIN DECOMPOSED
	mgm.	*mgm.*
Control	277	..
Bacterium No. 5 (crude culture)	190	87
Bacterium No. 5 (pure culture)	230	47
Fungus No. 2	238	39
Fungus No. 7	214	63

up to 1500 cc. with water. The nutrients were added, and the medium was distributed into flasks, which were sterilized, inoculated, and incubated for 25 days (table 7). In this experiment, as well, the various organisms were found capable of decomposing phenol-lignin.

Two cultures were now selected for testing the decomposition of the various phenol-lignin preparations, namely, a crude culture of a Fusarium and one of an Alternaria; ammonium phosphate and casein were used as sources of nitrogen. Acetone was employed as the solvent for the lignin and removed by evaporation under reduced pressure. The cultures were incubated for 40 days and the residual lignin determined (table 8). Some lignin was decomposed in all cases. This experiment was repeated using alcohol as a solvent for the lignin, since traces of acetone left in the medium were found to have a somewhat retarding effect upon the growth of the organisms. Much larger amounts of lignin were decomposed, both in the presence and in the absence of the solvent. It is of interest to note that in this experiment there was no increase in the resistance of the lignin to decomposition with an increase in maturity of the oat plant from which the lignin was isolated. This is

comparable to the behavior of the lignin in the native state, namely, in the oat plants, when the latter undergo decomposition.

In the final experiment, larger quantities of lignin were subjected to decomposition by two crude cultures of fungi. Six 8-gm. portions of phenol-lignin from the mature oat straw were dissolved in 200-cc. portions of alcohol and dispersed in 2 liters of water. The alcohol was removed by evaporation

TABLE 8

Decomposition of various lignin preparations by two crude cultures of soil microörganisms in acetone-free medium

NITROGEN SOURCE	ORGANISM	LIGNIN PREPARATION, MGM.									
		Oat plants, 59 days old		Oat plants, 86 days old		Oat plants, 112 days old		Lowmoor peat		Highmoor peat	
		Left	Decomposed	Left	Decomposed	Left	Decomposed	Left	Decomposed	Left	Decomposed
$(NH_4)_2HPO_4$	Control	141	..	159	..	189	..	133	..	133	..
	Fusarium	104	37	147	12	163	26	..	..	109	24
	Alternaria	120	21	147	12	167	22	112	21	108	25
Casein	Control	128	..	158	..	176	..	136	..	129	..
	Fusarium	108	20	107	51	164	12	63	73	93	36
	Alternaria	110	18	127	31	144	32	92	44	100	29

TABLE 9

Decomposition of phenol-lignin from oat straw by soil microörganisms

PERIOD OF DECOMPOSITION	CONTROL		FUSARIUM SP.			ALTERNARIA SP.		
	"Growth", weight	Residual lignin	"Growth", weight	Residual lignin		"Growth", weight	Residual lignin	
				Left	Decomposed†		Left	Decomposed
days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
30	0.926*	2.790	2.094	2.525	0.173	.....	.....	.....
98	.....	2.606	.....	.....	.....	1.791	2.201	0.497
230	.....	.....	1.151	2.171	0.527	1.882	2.019	0.679

* Consists almost entirely of casein, as shown by the fact that it contained more than 10 per cent nitrogen.

† Difference between average lignin content of culture and of the two controls.

of 400 cc. of the liquid under reduced pressure. All portions were combined and made up with water to 9600 cc.; 800-cc. portions of this lignin suspension were placed into 3-liter Fernbach flasks; each flask received 1 gm. of casein, 1 gm. K_2HPO_4, 0.5 gm. $MgSO_4$, and 0.01 gm. $FeCl_3$. Five flasks were inoculated with the crude culture of the Fusarium and five with the Alternaria (fungus No. 7); two flasks were left as controls. At the end of varying periods

of incubation at 28°C., the cultures were removed and acidified with HCl. The precipitate was either filtered off through paper or centrifuged, washed, and extracted with ethyl acetate. The insoluble residue was designated as "growth"; the soluble portion was evaporated, dried and weighed, and recorded as "residual lignin" (table 9).

Definite decomposition of the lignin took place, as shown by its gradual disappearance in the cultures. This was accompanied by the transformation of some of the casein nitrogen into microbial protein. The concentration of phenol was determined in the residual lignin and was found to be 15.88 per cent in the control and 15.67 per cent in the material upon which the fungus had grown, leading to the conclusion that the phenol was not split from the molecule and that the lignin complex was attacked as a whole. Although in the older cultures there was an increase in the soluble organic matter left in solution after the removal of the lignin, no definte lignin derivatives could be demonstrated. No acid formation could be detected.

DISCUSSION

Although in the decomposition of plant residues in composts and in soils the lignin constituents are the most resistant and tend to accumulate, they are not absolutely resistant and are attacked to a greater or lesser extent by a number of organisms. The nature of the plant material is of importance in this connection; the lignins in the straw of wheat and rye and in the leaves and needles of trees are much more resistant to decomposition than the lignins in alfalfa or in oat plants. This phenomenon, as well as the greater resistance of the lignin in mature than in young plants, has an important bearing upon the problem of formation and accumulation of humus. The results presented in this paper confirm similar observations already reported from this laboratory. However, a new fact was brought out here, namely, the significance of organic sources of nitrogen in the decomposition of the lignins in plant materials. This phenomenon also has a bearing upon such problems as mushroom production, where the organism in question feeds primarily upon the lignin derivatives in the compost.

An attempt was further made to decompose lignin isolated in a free state from the other plant constituents. In this respect, phenol-lignin proved to be very convenient, since it could easily be dispersed, by means of various solvents, in a liquid medium. Only in that state could it be subjected to decomposition. A number of common soil fungi were found capable of bringing about this process. In view of the fact that the important point was to demonstrate the capacity of soil organisms to attack isolated lignin, the specific nature of these organisms has not as yet been studied.

An attempt to determine the mechanism of the breakdown of the lignin complex failed. One can hope to do this only after the chemistry of lignin is better understood. The only facts that can be emphasized at present are the following: 1. Even those organisms that are capable of attacking isolated

lignin do it very slowly; 2. the lignin is utilized very efficiently, accompanied by the liberation of only very little CO_2, while the major part of the carbon is transformed into microbial cell substance; 3. the microbes utilizing lignin seem to build up cell substance which has an appreciable concentration of this complex. The second fact finds substantiation in the growth of mushrooms upon lignin-rich composts, whereas the third finds confirmation in the high lignin content of wood-destroying fungi (16). The problem has been further complicated by the fact that when alcohol was used as a solvent for the lignin, it could not be all removed from the dispersed medium; hence there is a possibility that some of the growth of the organisms may have been due to the utilization of the traces of alcohol left in the medium.

SUMMARY

A study has been made of the decomposition of lignin in a native state, namely, in oat plants harvested at different stages of growth, and of lignin isolated in a free state, in the form of phenol-lignin.

In accordance with results of other investigators and with those obtained in this laboratory, it was found that lignin in plant materials was more resistant to decomposition than the other groups of plant constitutents. However, it underwent a gradual, even if slow, decomposition.

Several organisms were isolated from the soil and found capable of decomposing phenol-lignin from spruce wood, oat straw, and peat. In order to bring about the decomposition of lignin by these organisms, it had to be dispersed in water. This was accomplished by dissolving the lignin in alcohol and adding it to an excess of water. The solvent was then removed by evaporation at reduced pressure.

Except in a few preliminary experiments, where crude enriched cultures of bacteria and fungi were employed, the rate of decomposition of the lignin was slow.

No product of lignin decomposition could be isolated or demonstrated.

The efficiency of the carbon utilization by the microörganisms attacking the lignin was very high.

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A COMPARISON OF THE SPECIFIC GRAVITY BALANCE AND THE PIPETTE METHODS OF DETERMINING DENSITY OF SOIL SUSPENSIONS

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In an experiment to determine the effect of cultural treatment of field soils on their permeability to irrigation water, it was thought desirable to make a study of the size distribution of individual soil particles and aggregates which remain more or less stable in a water suspension. This paper reports some tests on the use of a specific gravity balance as compared with the pipette for studying the size distribution as measured by settling velocities. A report of a previous investigation on dispersed soil in which the theory of the method has been discussed has been made by others.[1]

The balance used was lent to us for this purpose by Christian Becker, Inc. It (pl. 1) is an adaptation of their standard chainomatic balance especially designed for this purpose by mounting it on a stand with a rack and pinion to raise and lower the balance and plummet.

The procedure used in making a reading was to lower the plummet slowly into the liquid until its center of gravity was 15 cm. below the surface of the water. A rate of about 1 cm. per second was found to be a convenient speed. When lowered to the proper depth, the pointer was watched as the beam was lowered on the knife edge and was allowed to swing only far enough to indicate the direction of swing. The beam was then raised, the weights were readjusted, and the process was repeated until, when balanced, the pointer remained at zero. This static method was found to be necessary to prevent the plummet from moving up and down in the liquid and stirring it, which would interfere with the free falling of the particles in suspension. After each reading the plummet was raised out of the liquid at the same speed as that at which it was lowered. It was then rinsed with distilled water to clean it for the next reading. With a little practice it soon became easy to make readings in this manner.

A series of tests was made on dispersed soil. The results are shown in table 1. Columns 2, 3, and 4 show the percentages of soil suspension at the settling times shown in column 1. Ellipsoidal plummets, 10 cm. long, having the

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volumes indicated, were used. The means of the measurements obtained with plummets of this shape are shown in column 5. In column 6 are the results obtained with a spherical plummet. In column 7 are the results ob-

TABLE 1

Suspension densities at the end of various settling times, as measured by the specific gravity balance with different sized plummets and by the pipette method, 15 cm. below the surface

Densities expressed as per cent of the initial suspension density

TIME	ELLIPSOIDAL PLUMMETS OF DIFFERENT VOLUMES				SPHERICAL PLUMMET 10.15 cc.	PIPETTE
	46.35 cc.	14.14 cc.	4.53 cc.	Mean for ellipsoidal plummets		
min.						
1	56.38	58.18	56.60	57.72	34.95	56.85
3	50.60	52.00	49.30	50.63	47.20	50.20
5	44.40	46.80	44.70	45.30	44.83	45.85
10	37.32	39.70	37.20	38.07	40.10	38.70
20	33.15	33.96	32.60	33.23	33.60	33.10
30	29.82	31.50	29.54	30.28	29.65	31.15
hrs.						
1	26.25	25.49	24.04	25.26	24.83	26.15
2	22.03	22.32	19.42	21.26	22.77	22.50
4	18.40	18.84	18.16	18.47	19.68	19.55

TABLE 2

Replicate determinations on three soils of suspension densities 15 cm. below the surface, at various settling times, as measured by the specific gravity balance

Densities expressed as per cent of the initial suspension density

TIME	YOLO LOAM (SLACKED)				YOLO LOAM (DISPERSED)				SACRAMENTO ADOBE CLAY (SLACKED)				
	1	2	3	Aver-age	1	2	3	Aver-age	1	2	3	4	Aver-age
min.													
1	34.1	32.5	35.4	34.0	58.4	60.2	61.0	59.9	15.86	15.05	15.00	15.08	15.25
3	19.4	20.6	21.3	20.4	51.7	52.0	52.3	52.0	11.32	11.74	11.28	11.26	11.40
5	16.5	15.6	15.7	15.9	45.0	46.4	49.0	46.8	9.81	9.22	9.28	9.92	9.56
10	10.9	11.0	10.6	10.8	37.1	39.6	42.5	39.7	7.87	7.82	7.53	7.81	7.76
20	7.6	7.6	8.8	8.0	31.5	33.5	36.9	34.0	6.80	6.25	6.21	6.05	6.33
30	5.8	6.2	6.4	6.1	28.8	32.4	33.4	31.5	6.01	5.80	5.82	5.42	5.76
hrs.													
1	4.1	4.5	4.2	4.3	24.3	26.8	28.6	26.6	4.80	4.44	4.44	4.24	4.49
2	1.9	1.9	1.8	1.9	20.5	22.3	24.1	22.3	3.97	3.61	3.83	3.74	3.79
4					17.2	18.3	21.0	18.8					

tained with the pipette method. All readings were made at about 25°C. The center of each plummet was lowered to the same depth as that at which the pipette samples were taken. The difference between the buoyancies of the

suspension and of the pure water, together with the volume of the plummet, furnished sufficient information for a calculation of the average amount of material in suspension per unit of volume at the same depth as the center of the plummet. It was assumed that this represented the density of suspension at the depth where the center of the plummet was located. It is obvious that the settling velocity can be calculated from these data if desired.

TABLE 3

Suspension densities 15 cm. below the surface at various settling times as measured by the specific gravity balance and by the pipette

Densities expressed as per cent of initial suspension density

TIME	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette
min.										
1	74.9	82.3	71.7	76.7	75.0	81.1	77.6	81.9	74.8	76.7
3	70.8	70.6	66.3	63.3	72.0	68.0	69.7	64.7	70.7	67.2
5	63.6	64.2	59.8	59.6	64.8	62.6	62.4	59.9	62.9	59.3
10	53.5	55.0	52.8	51.6	56.5	54.5	55.4	52.6	55.0	53.5
20	46.8	46.3	45.1	47.6	47.5	45.4	47.0	45.8	47.4	46.5
30	41.8	44.1	40.7	41.9	42.7	42.3	43.3	41.1	43.0	42.0
hrs.										
1	35.6	36.3	34.5	36.0	35.8	34.8	36.7	35.8	36.7	36.0
8	20.3	22.3	21.3	23.1	21.7	21.0	22.9	23.0	22.4	23.3
24	14.3	19.0	15.2	19.1	15.7	17.4	17.9	18.0	16.7	18.0
48	11.0	14.5	12.2	16.0	12.4	15.2	13.8	15.0	13.5	15.5

	SAMPLE 6		SAMPLE 7		SAMPLE 8		SAMPLE 9	
	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette
min.								
1	75.6	77.8	57.4	61.1	75.3	80.4	44.6	48.1
3	70.4	68.1	49.2	59.4	75.1	77.7	42.5	39.7
5	69.4	61.6	45.2	45.6	73.4	76.4	38.0	34.2
10	56.2	55.1	38.9	40.3	70.0	73.4	32.6	29.8
20	47.6	47.3	34.7	35.0	66.7	70.2	27.4	25.0
30	43.3	43.8	32.2	33.2	64.4	67.5	25.3	24.2
hrs.								
1	38.7	37.7	27.6	28.2	60.9	64.6	20.8	21.3
8	22.0	24.8	17.5	19.1	54.9	55.7	13.2	14.2
24	15.6	19.6	13.3	15.2	49.5	50.3	10.7	9.35
48	11.9	15.8	10.8	12.0	47.4	48.5	9.6	9.30

The size of the ellipsoidal plummet within the range used here seems to make little difference in the readings obtained, and the data agree closely with those obtained by the pipette method. When the spherical plummet was used, material settled on the top of it while the first two readings were being taken. This caused these readings to be in error by a large amount. Later

readings, however, agree very well with the pipette results as well as with those obtained by the ellipsoidal plummets.

Another series of tests was run to determine how closely replicate determinations agreed. These results are presented in table 2. The tests were made on Yolo loam slacked in water, on Yolo loam dispersed with an electrical stirrer for 10 minutes without the aid of chemical dispersing agents, and on Sacramento adobe clay slacked in water. The reasonably good agreement among replicates is evident.

Since the pipette is usually regarded as the most accurate method of determining density of suspension, a further comparison of the balance and the pipette determinations on a number of soils was made. The results are given in table 3. The agreement here again is close.

TABLE 4

Suspension density 15 cm. below the surface at various settling times as measured by the specific gravity balance using suspensions having different initial suspension densities

Densities expressed as per cent of the initial suspension density

TIME	AIKEN CLAY LOAM			YOLO LOAM			SAN JOAQUIN SANDY LOAM		
	Per cent of concentrations			Per cent of concentrations			Per cent of concentrations		
	1	2	4	1	2	5	1	2	5
min.									
1	75.60	74.97	78.60	50.85	57.38	57.57	39.75	44.21	42.60
3	75.27	74.77	75.94	50.20	49.24	49.50	39.66	40.12	37.65
5	74.25	72.53	75.00	44.92	45.23	43.60	36.18	36.65	35.03
10	70.10	69.34	71.76	39.00	38.85	38.95	30.43	30.66	29.76
20	65.90	65.58	68.70	32.70	34.72	34.16	25.96	25.87	24.60
30	63.04	64.00	65.42	29.47	32.20	31.33	22.50	23.32	22.35
hr.									
1	59.42	60.20	61.72	25.22	27.60	27.18	20.18	19.72	18.90

It was recognized that the concentrations of the suspension might affect the determinations. The results of tests to determine whether this effect is appreciable are shown in table 4. Determinations at different concentrations on a given soil agree closely. Apparently concentrations as high as 5 per cent are not great enough to interfere with the free fall of particles.

The range of settling velocity which can be measured by this method is limited by the time required to make the first reading. One minute is about as soon as a reading can be made. This is also the case with the pipette method. It is difficult, therefore, to measure settling velocities greater than 0.25 cm. per second. A large part of the slacked soil had settled below the center of the plummet at the end of 1 minute, as shown in table 2. It is clear, therefore, that the balance is not suited for studying settling velocities greater than 0.25 cm. per second. The specific gravity balance is suitable for use over

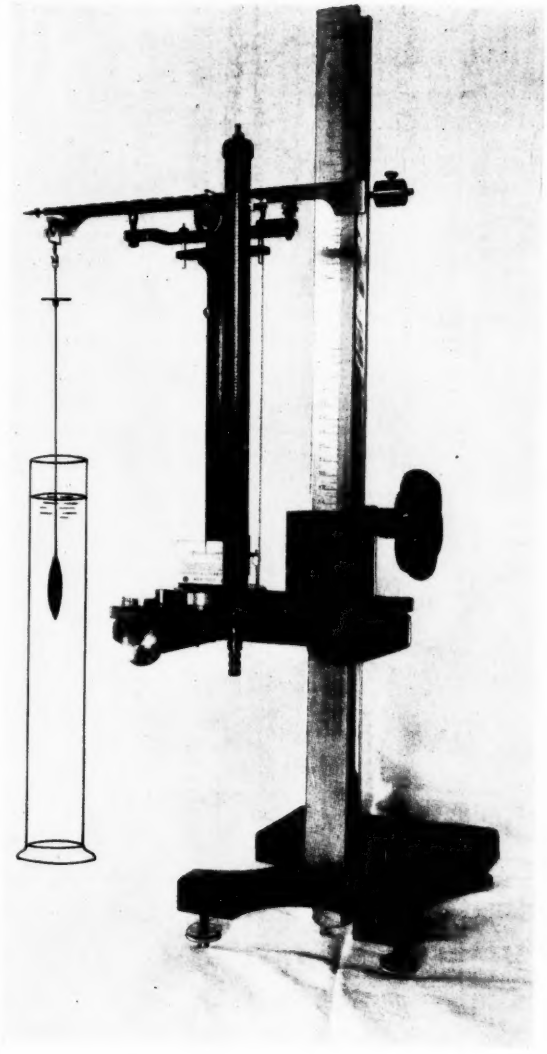
about the same range of settling velocities as the pipette method and requires considerably less working time to make the measurements.

SUMMARY

A comparison was made of the specific gravity balance and the pipette methods for determining the density of soil suspensions. Results obtained with different sized ellipsoidal plummets closely agree with one another and with the data obtained by the pipette method but do not agree with data obtained with a spherical plummet. Replicate determinations made with the specific gravity balance on a given soil agree closely. Initial suspension densities as high as 5 per cent were not great enough to interfere with the free fall of particles. The specific gravity balance is suitable for use over about the same range of settling velocities as the pipette method but requires considerably less time to make a measurement.

PLATE 1

SPECIFIC GRAVITY BALANCE USED IN MAKING SOIL SUSPENSION DENSITY DETERMINATIONS



SOIL SAMPLERS

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In studying some of the physical properties of soils, with particular reference to those influencing the movement and availability of water, it is often desirable to obtain soil samples the natural structure of which is undisturbed. Two types of cylinders have been devised and used with success by the writer in the soils of the Duke Forest.

Figure 1 shows a cylinder for obtaining samples of undisturbed soil for the determination of volume-weight, air space, and water-holding capacity. The unit consists of a cutting cylinder made of 5-inch high-pressure steel pipe machined down to the specifications indicated on the diagram and an inner cylinder made of 5-inch seamless brass tubing. The brass inner cylinder is held in place by a ring and pin while the unit is being driven into the soil with a sledge hammer. A counter-sunk steel plate or a block of wood placed on the cylinder prevents it from being battered by the hammer. The cutting edge should be case hardened. Constructed as indicated, the soil cylinder has a volume of 600 cc., a depth of slightly less than 2 inches, and an inside diameter of just under 5 inches.

Lids pressed from 10-ounce copper are used on the brass cylinders for transporting samples from the field to the laboratory. Squares of copper window screen, 16-mesh, have been used effectively over one end of the cylinders while the samples are being saturated with water, and later drained, for the determination of air space and water-holding capacity. Filter paper cut from paper toweling can be used to keep the soil from passing through the screen. Because of the great variation of volume-weight and allied measures of undisturbed soil, due to the presence of rocks, roots, and animal burrows, it is usually necessary to take a large number of samples in the field. The writer uses 50 brass inner cylinders with two cutting cylinders.

Figure 2 gives the dimensions of a cutting cylinder devised for taking samples of undisturbed soil for making determinations of the permanent wilting percentage. Cardboard ice-cream containers of one-pint capacity are placed in an inverted position in the cutting cylinder before it is driven into the soil. The bottom of the container is cut off in the greenhouse, and seeds of the plant to be used for determination of the wilting percentage are planted. The surface is not sealed until the plants have become well established and the moisture content of the soil has been reduced to near the wilting percentage. At that

time it is good to moisten the surface soil with water to a depth of 1 cm. and then seal the top of the container with paraffin to a depth of about 5 mm. The

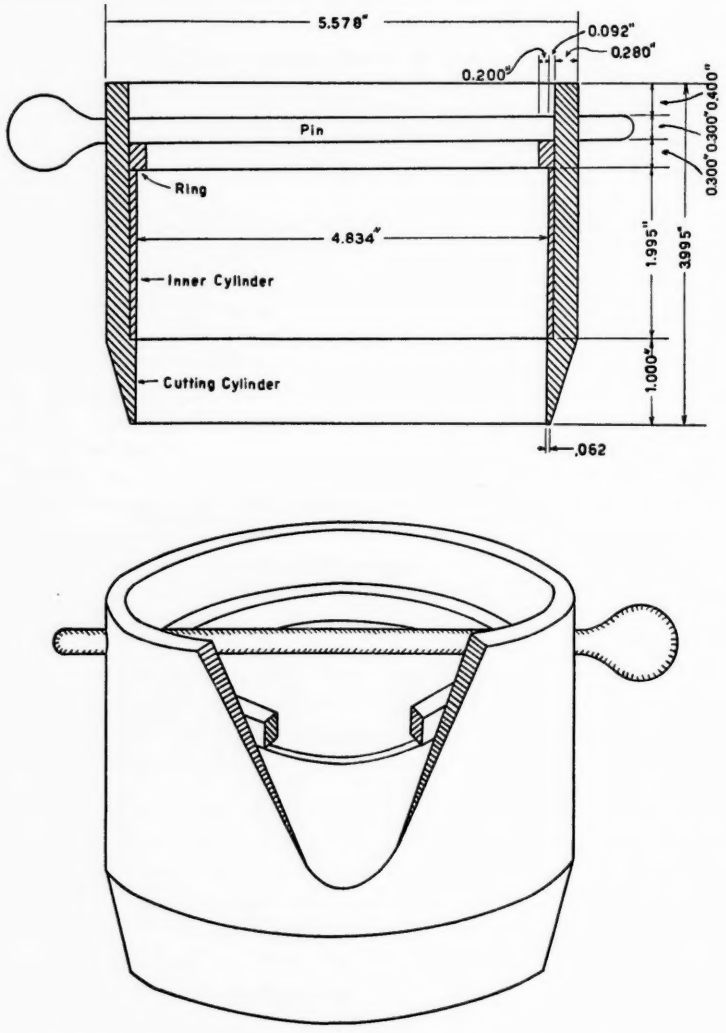

FIG. 1. SAMPLER FOR OBTAINING UNDISTURBED SOIL FOR DETERMINING VOLUME-WEIGHT, AIR SPACE, AND WATER-HOLDING CAPACITY

writer has found ordinary technical paraffin quite satisfactory when oats and sunflower are used as phytometers. The paraffin melts at about 54°C. If

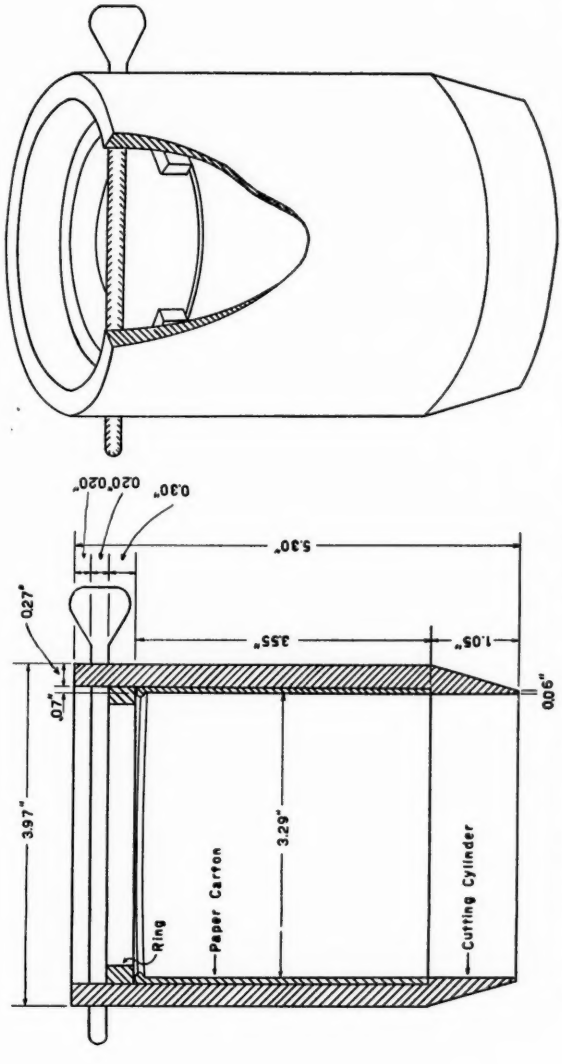

FIG. 2. SAMPLER FOR OBTAINING UNDISTURBED SOIL FOR DETERMINING THE PERMANENT WILTING PERCENTAGE

the temperature of the paraffin is not over 60°C. when it is applied it will solidify as soon as it contacts the moist soil and will not injure the plants. The containers can be boiled in paraffin before they are used and then coated on the outside after the field samples are obtained.

Either of the two cylinders described can be used for obtaining samples of undisturbed soil for moisture equivalent determinations. Sub-samples are cut out to fit the moisture equivalent cups in the laboratory.

TRANSFORMATION OF NITRATE IN WATER-LOGGED SOILS

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In comparison with the voluminous literature on the changes of nitrate in dry cultivated soils, that dealing with similar transformations in water-logged soils is limited. Among the early workers, Warington (16) was the first to notice that nitrate added to the soil could not be completely recovered when the soil remained covered with water. Nagaoka (9) observed that nitrites were formed as a result of heavy dressings of nitrate to rice fields. Daikuhara and Imasaki (2) have shown that denitrification takes place very rapidly in swamp soils, leading to the loss of nitrogen and to the formation of toxic nitrites. Kelley (8) has observed considerable fall in nitrate content in water-logged soils. Harrison and Aiyer (6), from an analysis of the gas evolved from swamp paddy soils, have concluded that the nitrate formed during the dry season is quickly denitrified and is of no use so far as the cultivator is concerned. More recently, Subrahmanyan (12) observed that water-logging resulted only in slight diminution in nitric nitrogen and that there was no formation of nitrite and no denitrification. Janssen and Metzger (7), on the other hand, found that in flooded soils nitrates are rapidly lost. Similar results were obtained by Prescott and Piper (10) who, working with Australian soils, observed rapid disappearance of nitrate at moisture contents above 18 per cent.

The foregoing review shows that although the previous workers were practically unanimous in their observation that water-logging of soil leads to considerable loss of nitrate, no attempt has been made to account for this loss, except the observations made by a few workers that denitrification takes place under conditions of water-logging.

The study of the changes of nitrate in water-logged soils is important from both the scientific and the practical points of view. No soil constituent shows such great fluctuations as nitrate and none is so susceptible to external influences. Under the widely differing chemical and biological conditions existing in water-logged soils, the course of the transformation of nitrate will probably be different from that in dry cultivated soils. From a practical point of view, the importance is even greater. Water-logging of soils, though comparatively less frequent in cold countries, is a common phenomenon in the tropics where millions of acres of dry land with the accumulated nitrate pass into the water-

[1] Thanks of the senior author are due to the Imperial Council of Agricultural Research for maintaining his salary.

logged state during rains. For the cultivation of rice, vast areas have to be maintained under swamp conditions for months. It is desirable, therefore, to obtain further knowledge of the effect of water-logging on the change of nitrate in soils; with this object in view the present investigation was undertaken.

METHODS OF ANALYSIS

Total nitrogen. In the absence of nitrate, total nitrogen was determined by the routine Kjeldahl method. A modification of Davisson-Parson's method (3) was used in estimating total nitrogen in water-logged soils containing nitrate. The soil-water mixture was distilled with 2 gm. of MgO and 2 gm. of Devarda's alloy, and the distillate was collected in 0.02 *N* H_2SO_4. The ammonia absorbed by acid was determined by back titration, using methyl red as indicator. The residue after distillation was digested with H_2SO_4 in the usual way. The sum of nitrogen in the first distillate and that in the residue, corrected for the nitrogen in the reagents used, gave the total nitrogen content of the soil.

Ammoniacal nitrogen was determined by Bengtsson's method (1). Nitric nitrogen was estimated by Devarda's alloy method.

Organic carbon was determined by the wet combustion method developed by Friedemann and Kendall (5). Correction was made for carbonate carbon where necessary.

Carbonate carbon was estimated by Collin's calcimeter.

Carbon dioxide evolved from soil was determined by absorption in 0.1 *N* $Ba(OH)_2$ solution and back titration against 0.05 N oxalic acid using phenolphthalein as indicator.

Soil organic matter. Analysis of soil organic matter was carried out according to the method developed by Waksman and Stevens (14).

Number of bacteria and actinomyces was determined by plating on Thornton's agar (13).

pH was determined by means of the quinhydrone electrode using soil-water mixture in the ratio 1:2.5.

SOIL SAMPLES USED

To observe the general effect of water-logging on nitrate, a few preliminary experiments using different soils were carried out. A known amount of nitrate was added to each soil, which was then water-logged and incubated. Considerable loss took place in every case. Out of this list, the following two, obtained from unmanured paddy fields, were selected for detailed study: Dacca farm soil—lateritic in type and acid in reaction (pH 5.1); Faridpur soil—a sandy loam alluvial soil alkaline in reaction (pH 8.3).

Samples of soil (0–6 inches) were air dried, passed through a 1-mm. sieve, and then preserved in bottles for use in the experiment.

EFFECT OF WATER-LOGGING ON THE NITRATE AND AMMONIA CONTENTS OF SOIL

By the addition of 100 cc. of distilled water in 250-cc. Erlenmeyer flasks, 50-gm. portions of soil were water-logged. The flasks were then divided into two sets: the first remained untreated and served as a control, and the second set received a known amount of nitrate as KNO_3. A third set of flasks each containing 15 gm. of soil and 100 cc. of distilled water, with or without added

TABLE 1

Changes in ammonia, nitrate, and total nitrogen in water-logged soil exposed to light

Results expressed as p.p.m.

Faridpur soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N		SOIL + 4.144 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	2.2	10.6	2.2	52.1	2.2	93.5
3	6.6	2.8	6.4	32.2	6.2	72.9
7	3.6	0.3	3.1	14.6	1.1	49.7
15	1.1	0.4	0.0	0.3	0.0	18.5
30	0.0	0.1	0.0	0.0	0.0	0.0
	Total N		Total N		Total N	
Start	639		680		722	
30 days	727		675		722	

Dacca soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	11.2	16.0	11.2	57.5
3	13.9	5.3	14.4	42.0
7	15.3	1.0	17.0	24.4
15	8.0	1.3	7.8	18.5
30	2.3	0.9	0.0	19.4
	Total N		Total N	
Start	961		1002	
30 days	951		989	

nitrate, was also set up and used for the determination of total nitrogen. The flasks were exposed to sunlight in the pot culture house. After 5 to 6 days' exposure, the evolution of gases from the soil surface was noticed in every flask. A few days later it was further observed that a greenish matter was appearing in the flasks containing Faridpur soil. This green growth gradually increased in bulk, and in a month the entire surface of the liquid was covered with a thick

growth of it. On the other hand, there was very little growth in the flasks containing Dacca soil. At intervals of 3, 7, 15, and 30 days, duplicate flasks from each treatment were removed for the determination of ammonia and nitrate. As nitrite was present only in traces, it was not estimated separately. Total nitrogen was determined only at the end of the experiment. The results are given in table 1.

TABLE 2

Changes of ammonia, nitrate, and total nitrogen in water-logged soil kept in the dark

Results expressed as p.p.m.

Faridpur soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	2.2	10.6	2.2	52.1
3	7.0	2.1	7.3	27.7
7	4.2	2.6	3.4	9.2
15	4.2	1.5	1.8	1.8
	Total N		Total N	
Start	639		680	
15 days	635		668	

Dacca soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	11.2	16.0	11.2	57.5
3	13.4	7.0	13.7	43.7
7	16.4	2.2	18.0	24.1
15	16.4	2.0	14.1	17.6
30	4.3	1.3	0.0	25.8
	Total N		Total N	
Start	961		1002	
30 days	946		986	

The appearance of the green algal growth in some of the flasks has introduced a complicating factor, since a portion of nitrate and ammonia might be used up for its body formation. To eliminate this factor, the experiment was repeated in the dark. The results (table 2) show that toward the latter part of the experiment a little ammonia and nitrate was assimilated by the growth.

The effect of water-logging, in general, is similar in both soils. Both have suffered heavy loss of nitrate and loss of ammonia after an initial rise but no great loss of total nitrogen. In spite of this resemblance, a difference will be

observed in their behavior when the figures for nitrate in the treated soils (soil + 2.072 mgm. NO_3–N) are compared. Whereas in Faridpur soil the added nitrate had nearly completely disappeared within the period of experiment, in Dacca soil, after a portion had been lost, no further reduction in the amount of nitrate took place; in fact, in the experiment in the dark, a rise from 17.6 p.p.m. on the fifteenth day to 25.8 p.p.m. on the thirtieth day was noticed. The behavior of Dacca soil is significant and shows that under certain conditions nitrate may survive in water-logged soils.

The loss of ammonia after an initial rise is in accord with the results of an investigation on the changes of ammonia in water-logged paddy soils carried out in this laboratory for three successive crop seasons. In all the 32 plots

TABLE 3

Effect of nitrate on the evolution of carbon dioxide from water-logged soil

Mgm. of carbon dioxide evolved from 300 gm. of soil

	0–2 DAYS	2–4 DAYS	4–7 DAYS	7–10 DAYS	10–13 DAYS	13–15 DAYS	15–18 DAYS
Untreated soil	10.2	18.1	54.9	47.1	30.5	29.2	21.2
Soil + nitrate (12 mgm. NO_3—N)	16.4	22.2	68.0	51.4	32.0	30.0	21.0

TABLE 4

Effect of nitrate on the numbers of bacteria and actinomyces in water-logged soil

Numbers of bacteria and actinomyces as millions, per gram of air-dry soil

DAYS AFTER WATER-LOGGING	BACTERIA AND ACTINOMYCES IN	
	Untreated soil	Soil + nitrate
3	3.75	6.0
7	5.75	12.0
10	3.45	6.0
18	2.55	2.5

selected for experiment, it was found that the ammonia content began to rise soon after water-logging, reached the maximum in about 6 weeks, thereafter remained stationary for about a month, and then fell. Similar observations were made in pots. In the foregoing experiments, the amount of nitrate lost was so much greater than the amount of ammonia formed that the reduction of nitrate to ammonia does not appear to be a reaction of importance in water-logged soils.

Except in control Faridpur soil (experiment in light), where there was a distinct increase, total nitrogen remained practically unchanged in all cases, the small differences observed being all within the limits of experimental error. As the results obtained at the end of the experiments, however, are consistently, though not significantly, lower than those at the start, it is believed that some

small loss of nitrogen occurred in the course of the experiment. But even then, the loss of nitrogen accounts for only a portion of the nitrate lost. The method for determining total nitrogen is not sufficiently accurate to detect losses of small amounts of nitrogen which may have occurred by dentrification. The increase in nitrogen in Faridpur soil is due to the fixation of atmospheric nitrogen by the green growth previously described (4).

From the foregoing observations, it would appear that the loss of nitrate in water-logged soils is not due to its reduction to ammonia, though a portion may be lost by denitrification. Evidently, the main loss is brought about by some other agency. It is presumed that nitrate is assimilated by microörganisms in the presence of the energy material supplied by the soil. If this view is correct, the loss of nitrate should be attended by a rise in bacterial numbers and an increased production of carbon dioxide. The results in tables 3 and 4 support this view. The experiment on which these results are based was performed with Dacca soil only, since the high content of carbonate in Faridpur soil made it unsuitable for use in an experiment on the production of carbon dioxide because of the absorption of the gas by the base, particularly during the early period of water-logging.

At first, carbon dioxide evolved in greater amounts from the treated soil, but the rate soon diminished until, after 13 days, nearly equal amounts were given off by the control and by the treated soils. Bacterial numbers also showed similar fluctuation. In the treated soil, the number was much higher in the beginning but after 10 days it dropped to the same range as that in the control. The rapid rise of bacteria for a few days in the beginning, followed by a decrease afterward, clearly explains why nitrate added to Dacca soil suffered loss only to 15 days and showed no further loss thereafter (tables 1 and 2). It is inferred that this slowing down of the bacterial activity is due to the exhaustion of the available energy supply of the soil.

ASSIMILATION OF NITRATE BY MICROÖRGANISMS IN RELATION TO SUPPLY OF ENERGY MATERIAL

As the supply of energy material is one of the main factors limiting the growth of the microörganisms, it is obvious that in a soil rich in energy material, nitrate will be assimilated to a greater extent than in one with a poor supply. The nearly complete disappearance of nitrate in Faridpur soil, compared with the limited loss in Dacca soil, indicates the relatively greater supply of energy in the former. If the excess of energy is reduced, it will probably behave in the same manner as the other, i.e., it will show only a limited loss of nitrate. This was tested in the following experiment.

A portion of Faridpur soil was incubated under optimum conditions of temperature and moisture until the maximum accumulation of nitrate took place. At this stage, the supply of nitrate was much in excess of the energy supply, otherwise the former could not have accumulated. The soil was then water-logged, and the changes of nitrate were observed. For comparison,

a portion of fresh soil (not incubated) was similarly water-logged after addition of nitrate and analyzed at stated intervals. The results are given in tables 5 and 6.

The influence of the energy materials on the transformation of nitrate in water-logged soil is clearly shown in table 6. In the fresh soil, containing much energy material, nitrate rapidly disappeared, until at the end of a month practically none was left. On the other hand, incubation resulted in the decomposition of much of the energy material; consequently the loss of nitrate in this case was small.

In view of the fact that under normal soil conditions the supply of energy material is a much more important limiting factor for the growth of microorganisms than is the nitrogen supply, it may be difficult to accept the hypothesis that nitrate added to water-logged soils is assimilated by the microörganisms. Table 5 shows that, in Faridpur soil, the quantity of nitrate had considerably increased within the first 15 days, indicating the presence of very

TABLE 5

Production of nitrate in Faridpur soil on incubation

Incubation period.....*days*	0	15	30	45	60	75*	90	105
NO_3—N.....*p.p.m.*	5.7	24.2	33.7	52.4	59.5	60.1	61.2	62.4

* After 75 days the incubated soil was water-logged.

TABLE 6

Effect of water-logging on the nitrate contents of fresh and incubated Faridpur soil

Incubation period.....*days*	0	3	7	15	30
NO_3—N in fresh soil.....*p.p.m.*	41.1	26.5	7.0	4.3	1.2
NO_3—N in incubated soil .....*p.p.m.*	60.1	57.6	55.1	53.1	48.1

little energy material in that soil, otherwise there would have been a lag in the accumulation of nitrate. On the other hand, results in table 1 and 2 show that in the same soil, when water-logged, more than 50 p.p.m. of nitrate was assimilated by the microörganisms in the same period, indicating that the soil had already contained a large excess of energy material. These two facts are apparently anomalous and require an explanation. It might be that a portion of the energy material, relatively less available in the dry soil, becomes easily available under the influence of water-logging, as a result either of hydrolysis or of greater solubility. In this connection it may be noted that to explain the rapid accumulation of nitrate in soils of Great Britain in late spring, Russell (11) assumed that "the winter frosts may cause a certain amount of physical disintegration of the organic matter and render some of it easily assimilable by the soil bacteria." Water-logging may bring about a similar change.

An experiment was next performed to observe the effect of the varying ratios between energy and nitrate on the assimilation of the latter by the microörga-

nisms. A culture solution[2] in 100-cc. lots was inoculated with 1-gm. portions of a soil previously water-logged; after incubation for 7 days at 33°C., total nitrogen was determined by the modified Davisson-Parson method. The results are given in table 7.

The results show that in the presence of sufficient energy material nitrate is rapidly assimilated by the microörganisms, from 30 to 50 parts of available carbon being necessary for assimilation of one part of nitric nitrogen, and that when the proportion of carbon is less than this amount a portion of nitrate is rapidly denitrified. Denitrification in soil must be an extremely slow process, for in none of the soils so far examined, not even in the incubated Faridpur soil in which the supply of energy is distinctly poor, did rapid denitrification similar to that observed in culture solution take place. In view of the fact that for microbial growth the conditions obtaining in culture solution are somewhat similar to those existing in water-logged soils, as far as the supply of air is concerned, it is permissible to advance the following hypothesis, on the basis of

TABLE 7

Effect of energy supply on the assimilation of nitrate

C:N	NITROGEN, IN MGM.		
	At start	After incubation	Gain or loss
5:1	6.86	1.87	−4.99
10:1	6.86	2.52	−4.34
20:1	6.86	4.67	−2.19
30:1	6.86	4.78	−2.08
50:1	6.86	7.60	0.74
60:1	6.86	8.0	1.14

the above results, regarding the change of nitrate in water-logged soils in relation to the supply of energy: In soils having ratios of energy carbon to nitrate nitrogen greater than 30–50:1, nitrate will be rapidly assimilated; when the ratios are lower, a portion of the nitrate will survive in the soil and may denitrify only very slowly. The validity of this hypothesis was next tested by examining the incubated and the fresh Faridpur soil.

A proximate method of analysis of the soil organic matter has been suggested by Waksman and Stevens whereby 90–95 per cent of all the constituents of the organic matter of the soil can be accounted for in groups of definite chemical complexes. It has been found by this method of analysis that soil organic matter is made up of the following groups: 1, Fats, waxes, and resinous bodies; 2, carbohydrates, including various hemicelluloses and the cellulose-like bodies; 3, lignin-humus complex; 4, proteins. It will be seen that group 2 includes

[2] K_2HPO_4—1.0 gm., $MgSO_4 \cdot 7H_2O$—0.2 gm., NaCl—0.1 gm., $FeCl_3$—trace, KNO_3—0.5 gm., $CaCO_3$—10.0 gm., Water—1,000 cc., glucose—varying amounts to correspond to different C:N ratios.

carbohydrates, which readily serve as energy material for microörganisms. This group, therefore, represents the available energy content of soil. Thus, if from the total organic carbon of soil, the sum of carbon included in groups 1, 3, and 4 is deducted, the balance will represent energy carbon of soil. It is admitted that the values obtained in this way can be, at best, only a rough approximation of the true energy content, but for the purpose of comparison between two soils in terms of available energy these figures might be taken without serious error as representing the relative energy contents of the soils.

The results of the analysis of the organic matter of Faridpur soil, both the fresh and the incubated, are given in table 8.

By subtracting from the carbon found in the sulfuric acid residue, the carbon of the nitrogenous complex, assuming that the latter is present as protein and contains 50 per cent carbon, the percentage of the lignin-humus carbon in the soil is calculated as follows: Per cent of lignin-humus carbon in the fresh soil $= 0.373 - \frac{0.037 \times 6.25}{2}$, or 0.257. Similarly the per cent of lignin-humus carbon in the incubated soil is 0.307. Protein carbon in the fresh soil $= \frac{0.062 \times 6.25}{2}$, or 0.194, and protein carbon in the incubated soil $= \frac{0.056 \times 6.25}{2}$, or 0.175. Neglecting the carbon present as fat, etc., the energy carbon is obtained by subtracting the sum of protein carbon and lignin-humus carbon from the total organic carbon: Energy carbon in the fresh soil = 640 − 451, or 189 mgm. in 100 gm. of soil. The ratio of energy carbon to nitrate nitrogen = 189:0.56 (table 5), or 337.5:1; similarly, in the incubated soil it is 90:6, or 15:1.

TABLE 8

Analysis of soil organic matter

Results in per cent

	FRESH SOIL	INCUBATED SOIL
Organic carbon	0.64	0.572
Organic nitrogen	0.062	0.056
Carbon in sulfuric acid residue	0.373	0.435
Nitrogen in sulfuric acid residue	0.037	0.041

If the fresh soil is treated with 4 mgm. of nitrate nitrogen, as in the first two experiments (tables 1 and 2), the ratio becomes 189:4.56, or 41.2:1, which is almost equal to the limiting ratio (lying between 30:1 and 50:1). Hence the added nitate should be rapidly assimilated in this case, and actually it was so. When Faridpur soil is treated with 8 mgm. of nitrate nitrogen per 100 gm. of soil (table 1), the ratio becomes 189:8.56, or 22:1. Nitrate should not have been completely assimilated in this case. But it should be pointed out that this experiment was performed in the light, and consequently a portion of the ni-

trate was used up by the green matter for its growth. The narrow ratio 15:1 of the incubated soil clearly explains why nitrate suffered only a slight loss in this case.

EFFECT OF ADDED ORGANIC MATERIALS ON TRANSFORMATION OF NITRATE

The use of a large quantity of green manure in the rice fields and the thorough incorporation, during puddling, of the heavy crop of weeds formed on the dry soil necessitate a study of the effect of the added materials on the transformation of nitrate in water-logged soils. Three plant materials, viz., rice straw, water hyacinth (*Eichhornia Crassipes*), and "Kalai" (*Phaseolus Mungo*, var Roxb.), were powdered, and portions supplying equal amounts of carbon were thoroughly mixed with 50-gm. portions of Faridpur soil, which were then water-logged and treated with nitrate. Ammonia, nitrate, and total nitrogen were estimated at intervals. The results are given in table 9.

TABLE 9

Change of nitrate, ammonia, and total nitrogen in the presence of added material

Results of ammonia and nitrate expressed as p.p.m.; total nitrogen, as mgm.

PLANT MATERIALS	TIME IN DAYS									
	0		3		7		15		30	
	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
Rice straw	2.2	45.4	5.8	0.3	0.3	0.3	0.3	0.3	0.6	0.3
Water hyacinth	2.2	45.4	21.6	0.3	3.2	0.6	55.7	0.1	94.1	1.0
Kalai	2.2	45.4	6.3	1.4	52.4	0.6	168.8	0.6	212.0	0.3
	Total N		Total N		Total N		Total N		Total N	
Rice straw	11.3		11.3		....		....		11.1	
Water hyacinth	23.6		23.6		....		....		21.4	
Kalai	21.7		21.7		....		....		17.1	

In every case nitrate quickly disappeared from the soil; the rate of disappearance was so rapid that even on the third day only a trace was present. The beneficial effect of green manure in water-logged soils thus appears to lie not only in supplying the nutrients to the crops but also in preventing the loss of nitrate by leaching. Waksman and Tenney (15) have shown that plant materials containing 1.7 per cent or more of N will undergo rapid decomposition without requiring the addition of extra nitrogen for that purpose. In view of this result, water hyacinth and Kalai should not have led to depletion of soil nitrate, as their own nitrogen (3.3 and 3.7 per cent respectively) is enough to meet the requirement of the microörganisms decomposing them. The rapid depletion of nitrate in this case suggests the possibility that microörganisms predominating under water-logged conditions may prefer nitrate to organic

nitrogen. As long as there was nitrate in the soil, the microörganisms may have attacked only the carbonaceous portions of the added materials for obtaining energy, the nitrogenous parts remaining largely unattacked during this period. After the nitrate was exhausted, the nitrogenous parts were then attacked, as shown by the rapid production of ammonia toward the latter part of the experiment. Soils treated with water hyacinth and Kalai lost a fair amount of nitrogen in a month. It is not understood how this loss was brought about. Similar loss of nitrogen has been noticed during the biological decomposition of organic materials of narrow C:N ratio (17, 18).

POT CULTURE EXPERIMENT

In order to get an idea of the magnitude of loss of nitrate from soil by bottom drainage, both in the presence and in the absence of a crop, a pot culture experiment was set up in which a known amount of nitrate was added to each of a

TABLE 10

Loss of nitrate from water-logged soil in drainage water

POT NUMBER*	MGM. OF NITRIC NITROGEN RECOVERED									
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	Total
1	....	....	....	....	...	...	...	...	...	....
2	4.5	4.1	0.1	....	...	...	...	...	...	8.7
3	6.2	13.9	0.1	....	...	...	...	...	...	20.2
4	20.1	24.1	16.5	11.5	5.2	1.1	...	...	...	78.5
5	10.0	26.4	24.9	16.0	9.6	5.5	1.6	...	...	94.0
6	1.6	0.4	0.3	0.1	...	...	...	...	...	2.4

* Pot 1—unmanured but cropped; pots 2 and 3—manured and cropped; pots 4 and 5—manured but uncropped; pot 6—unmanured and uncropped.

number of pots and the leachate from each pot was collected and analyzed daily until it was found free from nitrate. The experimental details are as follows:

The experimental unit was a glazed earthenware pot (8 inches by 12 inches) with a single tubulure at the bottom of the side. Each was fitted with a delivery tube and mounted on a stand so as to allow a flask standing below it to receive drainage from the delivery tube. Six such pots were prepared, and a 2-inch layer of sand was put in each. A sample of Dacca Farm soil, sifted through a coarse sieve and thoroughly mixed, was fed into the pots 8 pounds at a time with gentle pressing until each pot contained 32 pounds of soil. The pots were then water-logged and allowed to rest for 3 months. Rice seedlings were transplanted into three of the pots, and water was drained through the delivery tubes daily. The soil in the pots was always kept water-logged by the periodic additions of distilled water. Before ear emergence, nitrate was applied as KNO_3 to four pots at the rate of 222 mgm. of nitric nitrogen per pot. The drainage was regulated in such a way that about 500 cc. of the drainage

water accumulated from each pot in 24 hours. After every 24 hours the receivers were changed and the nitrate leached out from each pot was determined. Even after nitrate had disappeared from the drainage water, analysis was continued for a few days more to ensure that nitrate had been completely washed out of the pots.

The results show that about 40 per cent of the added nitrate was recovered in the drainage water from the uncropped pots, but from the corresponding cropped pots the amount recovered was only 7 per cent.

SUMMARY

Experiments performed with a number of soils showed that nitrate is rapidly lost under water-logged conditions.

Detailed experiments with Dacca and Faridpur soils have shown that this loss is not due to the reduction of nitrate to ammonia; neither is there evidence to show that in soils under water-logged conditions all of the nitrate is denitrified.

An increased production of carbon dioxide and a rise in bacterial numbers occur in water-logged soil after addition of nitrate, showing that the added nitrate is assimilated by the microörganisms.

It has been suggested that, in soils having ratios of energy carbon to nitrate nitrogen greater than 30–50:1, nitrate will be rapidly assimilated by the microorganisms when such soils are water-logged; when the ratios are narrower, a portion of the nitrate will remain in the soils and may denitrify slowly.

In the presence of added organic materials, whether rich or poor in nitrogen, nitrate disappears very quickly.

A pot culture experiment showed that in the presence of a rice crop, little nitrate is lost in drainage water; in the absence of a crop, the loss is considerable.

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TOTAL NITROGEN AS A FACTOR INFLUENCING NITRATE ACCUMULATION IN SOILS[1]

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Although 50 pounds of nitrogen are required to produce an average acre grain crop and cultivated soils usually contain from 1000 to 4000 pounds of nitrogen in the surface foot, nitrogen is the nutritive element most frequently limiting plant growth in cultivated soils. These apparent contradictions arise from the fact that the soil's store of nitrogen is largely in a nonavailable form and becomes available rather slowly, particularly in soils that have been long under cultivation.

Because of the facts just stated, students of soil fertility have been much interested in the factors influencing the conversion of nonavailable nitrogen into an available form. Among the significant factors determining the ability of a soil to supply an adequate quantity of available nitrogen to a growing crop, the total quantity of combined nitrogen in the soil has been regarded as of paramount importance. Many data are on record indicating a direct relationship between the quantity of combined nitrogen in a soil and the ability of the soil to accumulate nitrate nitrogen during a limited period. There are, however, enough exceptions to this relationship to suggest that other factors may frequently be of equal or greater importance. In no instance, to the writer's knowledge, have data developed in such a study been submitted to statistical treatment to determine mathematically the significance of the apparent relationship. It is the purpose of this paper to present a limited study of this nature.

PRESENTATION OF DATA

Some years ago the writer (2) directed attention to a specific instance wherein the ability of a soil to accumulate nitrate nitrogen rapidly under laboratory conditions appeared to be more intimately associated with a certain small portion of its store of nitrogen than with the total quantity. In the accompanying discussion attention was directed to a suggestion made by Lawes, Gilbert, and Warrington (5) that the major portion of the store of nitrogen in a long-cultivated soil appears to be inert and that in such a soil the ability to supply

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a growing crop with adequate nitrogen depends, in part at least, upon the annual acquisition of a new supply of more easily nitrifiable nitrogen.

During the past few years a sufficiently large number of conditions somewhat similar to that noted above have been observed among soils studied to prove that the suggested explanation actually obtains in the field. The data that served as a basis for this conclusion are being presented elsewhere in the form of a detailed study of the rôle played by nitrogen in the production of "fertile" spots in 165 wheat fields (4). The term "fertile" as here used should apply literally only when a particular "fertile" sample is compared with its corresponding "nonfertile" sample. The "fertile" samples of soil were in all cases collected from small areas of soil such as previously pictured and described (3) as appearing in fields of small grain, and the "nonfertile" samples were collected from the area immediately surrounding the spots. Such spots frequently appear in fairly productive soils, hence the "nonfertile" of a pair of samples in one instance may have come from a much more highly productive soil than the "fertile" sample in another pair.

In the study referred to it was found that, in general (91 per cent of the comparisons), soil from the "fertile" spots possessed a more marked ability (on the average 3+ times) to accumulate nitrate nitrogen from its store of nitrogen than did the "nonfertile" sample collected only a few feet away. A statistical study of the data led to the conclusion that the more marked nitrate accumulating ability of the "fertile" soil was very definitely correlated with the presence of a small excess of total nitrogen over that present in the "nonfertile" soil. On the average this difference amounted to only 120 p.p.m. nitrogen. It was shown, though, that this 120 p.p.m. of nitrogen was capable of being converted into nitrate nitrogen more than 27 times as rapidly as was the other 1390 p.p.m. present in the "fertile" or in the corresponding "nonfertile" sample.

There are, no doubt, many soils that contain exactly the same quantity of combined nitrogen, but probably in no two soils, or two samples of the same soil, is this nitrogen present in the same forms. If, as has just been indicated, a small fraction of the nitrogen can play such an important rôle in the ability of the soil to accumulate nitrate nitrogen, and the combinations in which nitrogen exist in soils are so numerous, the question naturally arises: To what extent may such factors alter the influence of the total nitrogen as a factor controlling the nitrate accumulating abilities of soils?

In the investigation already referred to, the nitrate accumulating ability of 125 pairs ("fertile" and "nonfertile") of soils, or a total of 250 individual samples, was determined. These soils were also analyzed for total nitrogen, thus making possible a study of the relationship between the two factors. In no instance were soils selected on a basis of their probable nitrogen contents, yet they varied widely in this respect, being rather uniformly distributed over the range from 500 to 2000 p.p.m.

The details as to methods of collecting, handling, and analyzing the soils are being presented elsewhere (4) and need not be repeated here. Suffice it to

TABLE 1

Total nitrogen content and nitrate accumulating ability of 124 "nonfertile" soils

SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
1	330	44	43	1,310	99	85	1,530	110
2	380	33	44	1,320	151	86	1,540	156
3	550	134	45	1,320	17	87	1,550	181
4	560	71	46	1,320	59	88	1,560	111
5	600	71	47	1,330	145	89	1,560	96
6	610	36	48	1,330	102	90	1,570	55
7	740	83	49	1,340	139	91	1,570	80
8	760	54	50	1,350	78	92	1,570	47
9	770	71	51	1,350	193	93	1,590	114
10	960	52	52	1,360	39	94	1,590	185
11	960	142	53	1,360	138	95	1,600	88
12	1,010	87	54	1,370	88	96	1,600	194
13	1,030	75	55	1,370	34	97	1,610	142
14	1,030	70	56	1,370	198	98	1,630	78
15	1,030	91	57	1,380	101	99	1,660	164
16	1,030	140	58	1,380	57	100	1,660	181
17	1,050	136	59	1,380	133	101	1,670	92
18	1,080	66	60	1,390	154	102	1,670	43
19	1,080	95	61	1,400	342	103	1,690	270
20	1,100	132	62	1,400	128	104	1,700	75
21	1,110	132	63	1,400	141	105	1,700	59
22	1,120	97	64	1,410	138	106	1,710	221
23	1,120	149	65	1,410	98	107	1,720	181
24	1,130	44	66	1,410	81	108	1,730	147
25	1,130	61	67	1,420	152	109	1,800	83
26	1,140	99	68	1,430	195	110	1,810	85
27	1,150	131	69	1,430	52	111	1,810	195
28	1,160	77	70	1,440	44	112	1,820	101
29	1,180	149	71	1,440	177	113	1,830	101
30	1,200	41	72	1,440	189	114	1,840	111
31	1,220	67	73	1,450	79	115	1,860	232
32	1,220	85	74	1,460	120	116	1,860	128
33	1,230	63	75	1,460	73	117	1,880	60
34	1,240	151	76	1,470	91	118	1,890	280
35	1,240	102	77	1,470	92	119	1,890	208
36	1,250	75	78	1,470	193	120	2,060	158
37	1,270	61	79	1,470	72	121	2,110	182
38	1,270	45	80	1,490	219	122	2,150	164
39	1,270	184	81	1,500	71	123	2,160	166
40	1,290	248	82	1,500	176	124	2,300	215
41	1,290	89	83	1,510	59			
42	1,290	60	84	1,510	75			

Correlation coefficient 0.389 ± 0.052.
Average nitrogen content 1390 p.p.m.
Average NO_3 content 116 p.p.m.

TABLE 2

Total nitrogen content and nitrate accumulating ability of 125 "fertile" soils

SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
1	360	−8	43	1,400	647	84	1,660	371
2	440	128	44	1,400	201	85	1,660	320
3	610	168	45	1,410	213	86	1,660	340
4	610	85	46	1,420	238	87	1,670	554
5	610	108	47	1,440	373	88	1,680	125
6	770	105	48	1,450	62	89	1,680	251
7	810	97	49	1,450	214	90	1,700	1,028
8	870	142	50	1,450	520	91	1,710	633
9	920	559	51	1,460	121	92	1,720	461
10	990	77	52	1,460	847	93	1,730	247
11	1,010	219	53	1,460	309	94	1,740	89
12	1,020	141	54	1,480	143	95	1,760	330
13	1,030	116	55	1,480	79	96	1,770	409
14	1,040	101	56	1,490	462	97	1,770	295
15	1,080	404	57	1,500	466	98	1,780	1,200
16	1,100	118	58	1,500	177	99	1,780	373
17	1,130	190	59	1,500	55	100	1,810	413
18	1,140	154	60	1,510	1,126	101	1,820	156
19	1,150	524	61	1,510	758	102	1,820	515
20	1,190	71	62	1,520	1,086	103	1,840	136
21	1,200	445	63	1,530	324	104	1,870	782
22	1,250	64	64	1,540	193	105	1,870	254
23	1,250	632	65	1,540	274	106	1,870	1,418
24	1,260	316	66	1,550	189	107	1,880	223
25	1,270	147	67	1,550	161	108	1,880	611
26	1,280	52	68	1,560	414	109	1,890	430
27	1,290	283	69	1,570	368	110	1,910	98
28	1,300	348	70	1,570	201	111	1,920	302
29	1,300	507	71	1,580	112	112	1,950	546
30	1,300	188	72	1,580	779	113	1,950	537
31	1,310	267	73	1,580	382	114	1,950	875
32	1,350	610	74	1,580	889	115	1,970	741
33	1,350	80	75	1,590	111	116	1,980	471
34	1,360	778	76	1,590	234	117	2,020	249
35	1,360	255	77	1,610	101	118	2,020	349
36	1,380	200	78	1,610	597	119	2,080	546
37	1,380	69	79	1,620	250	120	2,140	724
38	1,380	986	80	1,630	1,571	121	2,180	305
39	1,380	96	81	1,630	171	122	2,210	1,081
40	1,380	244	82	1,630	538	123	2,210	447
41	1,390	380	83	1,650	186	124	2,310	769
42	1,400	212				125	2,310	332

Correlation coefficient 0.368 ± 0.052.
Average nitrogen content 1510 p.p.m.
Average NO_3 content 375 p.p.m.

say that all samples were treated alike in that they were made up to optimum moisture content and incubated at room temperature 4 to 6 weeks, after which the nitrate (NO_3) content was determined. The initial NO_3 was deducted from that present after incubation, and the net gain thus obtained was taken as a measure of the nitrate accumulating ability of the sample.

The values obtained, by the afore-described procedure, from the "nonfertile" samples are presented in table 1, and those from the "fertile" samples, in table 2. In both tables the soils are arranged in the order of increasing nitrogen contents, hence the corresponding numbers in the two tables do not represent pairs of soils collected from the same field. In general, though, the paired samples will fall in the same group in table 3, where the data are summarized, the samples of similar nitrogen contents being grouped together and only the average nitrogen and nitrate values for these groups recorded.

TABLE 3

Average nitrogen content and nitrate accumulating ability of soils

RANGE IN NITROGEN CONTENT OF SOILS	"NONFERTILE" SERIES			"FERTILE" SERIES		
	Number of soils in group	Average nitrogen content	Average NO_3 formed	Number of soils in group	Average nitrogen content	Average NO_3 formed
p.p.m.		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
0 to 1,000	11	656	72	10	699	146
1,001 to 1,200	19	1,098	98	11	1,099	226
1,201 to 1,400	33	1,321	114	23	1,336	329
1,401 to 1,600	33	1,498	117	32	1,513	371
1,601 to 1,800	13	1,688	134	23	1,689	454
1,801 to 2,000	10	1,849	150	17	1,893	501
Above 2,000	5	2,156	177	9	2,164	534
Average.....		1,390	116		1,510	375

Correlation coefficient based on group averages: "nonfertile" series 0.990 ± 0.012; "fertile" series 0.988 ± 0.006.

DISCUSSION

A study of the data presented in table 3 alone could lead to but one conclusion, i.e., a very close and direct relationship exists between the nitrogen content of these soils and their ability to accumulate nitrates. A similar conclusion would be reached by a study of the oft quoted average values presented by Fraps (1). Furthermore, this conclusion apparently would be proved sound by a calculation of the correlation coefficients for the two sets of data recorded in table 3 and for the general average values recorded by Fraps, in that the almost perfect coefficients of 0.988 ± 0.006, 0.990 ± 0.012, and 0.995 ± 0.003 are obtained respectively for the "fertile," "nonfertile," and Fraps' data. More nearly perfect correlation values could not be wished for.

Almost equally convincing relationships are sometimes obtained from a study of a limited number of more or less selected individual soils. For example,

among the few soils studied by Sievers and Holtz (7) there is no exception to the rule that, as the nitrogen content of the soil increases, its ability to accumulate nitrates increases, and the correlation coefficient calculated from these data, 0.921 ± 0.057, approaches perfection.

On the other hand, when the data presented in tables 1 and 2 are studied in detail it becomes evident that there are a very large number of irregularities in the direct relationship between total nitrogen and nitrate accumulation. Similar marked irregularities are present in other instances where large numbers of soils, taken at random, have been studied; for example, in the data presented by Lipman, Burgess, and Klein (6).

The presence of so many marked exceptions in the relationship in any series could not help but influence materially the mathematical interpretation of data that is smoothed out by averaging results. In this instance it is found that the nearly perfect correlations obtained from the average values become almost negligible when calculated upon the individual samples. The 0.988 ± 0.006 value for the "fertile" series becomes 0.368 ± 0.052, and the 0.990 ± 0.012 for the "nonfertile" series becomes 0.389 ± 0.052; in both instances very little, if any, correlation is indicated. Since Fraps failed to record the data for the individual samples, similar comparisons cannot be made with his studies.

These correlation coefficients were so unexpectedly low in view of the close relationship shown by averaging data for the several groups that it seemed desirable to make a similar comparison with data obtained elsewhere. Because of the large number of soil samples involved, the data presented by Lipman, Burgess, and Klein (6) were chosen, and their humid and arid groups have been treated separately. Because of the limited number of humid samples, 47, they were divided into only five approximately equal groups on a basis of total nitrogen content and the average nitrogen content and nitrate accumulation for the various groups determined. By using the average values thus obtained as a basis for calculating the correlation between the two factors, the fairly significant value 0.727 ± 0.142 is obtained. The corresponding value calculated on a basis of the individual samples is 0.479 ± 0.073.

Similarly, the 140 arid soil samples were divided into groups on the basis of differences of 200 p.p.m. nitrogen up to 1600 p.p.m. and, because of the limited number, samples containing in excess of 1600 p.p.m. thrown into a single group, thereby making 9 groups each of which contained from 7 to 37 samples. When the average nitrogen and nitrate values of these groups were used as a basis for calculating the correlation coefficient, the value 0.686 ± 0.119 was obtained. A similar calculation based upon the 140 individual samples gave a coefficient of only 0.190 ± 0.037. Other comparisons with comparable results have been made. It would thus appear that the relationships found to exist between the nitrogen content and the nitrate accumulating ability of these Kansas soils are of the same order as similar relationships based upon data reported by other investigators.

The object in calling attention to the facts presented in the preceding pages has not been to discredit the value of total nitrogen as a factor limiting the

accumulation of nitrates in soils, but rather to suggest the possibility of arriving at erroneous conclusions when data of this nature are loosely interpreted by averaging values, as has been rather freely done in the past.

The fact should not be lost sight of that these studies have been based upon a relatively short incubation period. It is difficult to see how the total quantity of nitrogen could help but be a major limiting factor in nitrate accumulation when the time factor is eliminated.

From a practical point of view, the ideal rate of nitrate formation should be just sufficient to prevent nitrogen from becoming the limiting factor in plant growth. In the studies already referred to, it is shown that, in the "nonfertile" soils here discussed, nitrogen was a major limiting factor in the growth of the wheat plant. The presence of the 120 p.p.m. additional nitrogen in the fertile soil largely eliminated this condition, not because of the additional nitrogen alone, but because it was in such a form that it could be more readily changed into an available condition. So long as the quantities of easily nitrifiable nitrogen vary in soils it cannot be expected that a perfect correlation will exist between nitrogen content and nitrate accumulation, at least during a limited incubation period. The greater the variation in the quantities of such forms of nitrogen, the less marked will be the direct relationship between the two factors. Apparently such variations are sufficient in Kansas soil that, coupled with other factors, the influence of total nitrogen upon nitrate accumulation may be almost completely overshadowed during relatively short periods of time.

SUMMARY

When the nitrogen content and the nitrate accumulating abilities of a large number of soils are determined and the data thus obtained are grouped on a basis of the nitrogen content of the soils and averaged, an almost perfect direct relationship may appear to exist between the total nitrogen content and the nitrate accumulating ability. On the other hand, if the original data are used as a basis for calculating the coefficient of correlation, the relationship between the two factors may be found to be very slight, or even nil.

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